



GRAS Notice (GRN) No. 501

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

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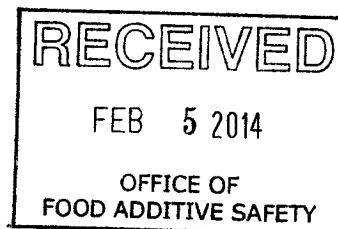
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浙江天瑞化学有限公司
ZHEJIANG TIANRUI CHEMICAL CO., LTD.
GRN 000501

January 27, 2014

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835



Dear Sir or Madam,

Zhejiang Tianrui Chemical Co., Ltd. (Tianrui Chemical hereafter) is hereby submitting for consideration a information supporting the use of high-purity L-theanine ingredient, also referred to as *L-theanine* ($\geq 98\%$), in human foods is generally recognized as safe (GRAS), as shown through scientific procedures.

L-Theanine ($\geq 98\%$) is chemically-synthesized using food-grade materials and conventional GMP food industry processes to meet strict established specifications. It is intended to be used in select foods for the general human population at levels up to 250 milligrams per serving (250 mg/serving).

To make the GRAS determination, Tianrui Chemical relied largely on information derived from two prior documents filed by U.S. FDA as GRN No. 209 submitted by Taiyo International, Inc. and GRN No. 338 submitted by Blue California. In both cases, FDA indicated based on the available information that it had no questions regarding each company's conclusion that L-theanine is GRAS under the intended conditions of use, as an ingredient in fruit juices and drinks, non-herbal teas, sports beverages, bottled waters, chocolate bars and chews, hard candies, breath mints, and chewing gum at levels up to 250 mg per serving.

Since Tianrui Chemical intends to use *L-Theanine* ($\geq 98\%$) in the same foods and at the same levels as the two prior notices, the present notice relies on these documents as the basis for the conclusion that the use of *L-Theanine* ($\geq 98\%$) is likewise GRAS. Tianrui Chemical's *L-Theanine* ($\geq 98\%$) is different in that it is chemically-synthesized from L-glutamic acid and ethylamine, rather than being derived through enzymatic action (GRN No. 209) or extracted from tea leaves (GRN No. 338).

Details about the manufacturing method, specifications, and quality control procedures are provided in the attached document, along with various excerpts from the two prior GRAS notices that are considered relevant to a discussion about the safety of Tianrui Chemical's L-theanine ingredient.

Please note that, in addition to Tianrui, the following individuals from Intertek Cantox, located at 1011 U.S. Highway 22, Suite 200, Bridgewater, NJ 08827, have been authorized by Tianrui Chemical to engage in discussions about the present GRAS Notice.

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浙江天瑞化学有限公司

ZHEJIANG TIANRUI CHEMICAL CO.,LTD.

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Sincerely,

(b) (6)

Signature: _____

Date: _____

Jan. 27, 2014

Zhang Sheng

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GRAS ASSESSMENT

L-Theanine ($\geq 98\%$)

January 27, 2014

Zhejiang Tianrui Chemical Co., Ltd.

No.12 Xingye Road, South Industrial Zone,

Longyou County, Zhejiang Province, China 324400

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)¹

Zhejiang Tianrui Chemical Co., Ltd. (Zhejiang Province, China), also referred to as Tianrui Chemical, has determined that the use of its manufactured ingredient, *L-theanine* ($\geq 98\%$), a high-purity L-theanine, in food for humans is generally recognized as safe (GRAS) in accordance with Section 201(s) of the federal food, Drug, and Cosmetic Act. This determination is based on scientific procedures, as described in the following sections, and the evaluation accurately reflects the intended food uses of *L-theanine* ($\geq 98\%$).

(b) (6)

Signature _____
Zhang Sheng
General Manager

Date Jan. 27, 2014

B. Name & Address of Notifier

Zhejiang Tianrui Chemical Co., Ltd.
No.12 Xingye Road, South Industrial Zone,
Longyou County, Zhejiang Province, China 324400

Contact Name: Zhang Sheng
Phone: +86-570-7222999
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E-mail: zjtrchem@VIP.163.com

As the notifier, Tianrui Chemical accepts responsibility for the GRAS determination for *L-theanine* ($\geq 98\%$), as described in the subject notification. Consequently, Tianrui Chemical's *L-theanine* ($\geq 98\%$) ingredient, containing greater than or equal to 98% L-theanine and meeting the conditions described therein, is exempt from the pre-market approval requirements for food ingredients.

¹ See 62 FR 18938 (17 April 1997), which is accessible at <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm>

C. Common Name & Identity of the Notified Substance

L-Theanine (frequently shortened to theanine) is the common name for the notified substance. The specific material of interest to Tianrui Chemical is a high-purity ($\geq 98\%$) L-theanine, also referred to as *L-theanine* ($\geq 98\%$).

D. Conditions of Intended Use in Food

L-Theanine ($\geq 98\%$) is intended to be added as a nutrient supplement into a limited number of conventional food categories described in this document.

E. Basis for the GRAS Determination

Pursuant to 21 CFR §170.30, the use of *L-theanine* ($\geq 98\%$) in food for humans has been determined to be GRAS on the basis of scientific procedures. A summary of the information upon which the GRAS determination is based is provided in the attached GRAS dossier.

A search of the U.S. FDA inventory of GRAS notices² for “theanine” revealed two prior documents filed as GRN No. 209 submitted by Taiyo International, Inc. and GRN No. 338 submitted by Blue California. In both cases, FDA indicated based on the available information that it had no questions regarding each company’s conclusion that L-theanine is GRAS under the intended conditions of use, as an ingredient in fruit juices and drinks, non-herbal teas, sports beverages, bottled waters, chocolate bars and chews, hard candies, breath mints, and chewing gum at levels up to 250 mg per serving.

Since Tianrui Chemical intends to use *L-Theanine* ($\geq 98\%$) in the same foods and at the same levels as the two prior notices, the present notice relies on these documents as the basis for the conclusion that the use of *L-Theanine* ($\geq 98\%$) is likewise GRAS. Tianrui Chemical’s *L-Theanine* ($\geq 98\%$) is different in that it is chemically-synthesized from L-glutamic acid and ethylamine, rather than being derived enzymatically (GRN No. 209) or extracted from tea leaves (GRN No. 338).

F. Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to the US Food and Drug Administration (FDA) upon request, or will be made available for review and copying at reasonable times at the offices of Intertek Cantox, located at 1011 U.S. Highway 22, Suite 200, Bridgewater, NJ, USA 08807.

² GRAS Notice Inventory available online through:

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>.

II. INTRODUCTION

A. Objective

With the assistance of Intertek Cantox (Bridgewater, NJ), an independent consulting firm specializing in product safety and regulatory affairs, Zhejiang Tianrui Chemical Co., Ltd., also known as Tianrui Chemical, has undertaken an independent safety evaluation of its *L-theanine* ($\geq 98\%$) or high-purity L-theanine ingredient. The purpose of the evaluation was to ascertain whether the use of *L-theanine* ($\geq 98\%$) as a nutrient supplement in selected food products for the general human population might be considered generally recognized as safe (GRAS).

B. Foreword

In the present GRAS dossier, Zhejiang Tianrui Chemical Co., Ltd. provides detailed information about the intended foods and use levels, ingredient manufacturing, specifications, and batch analyses, along with a summary discussion of the safety of theanine. Determining how much theanine may be safely consumed, *i.e.*, the so-called “dose” or use levels, is critical in the determination of safe exposure levels for theanine when consumed as a food ingredient. The composite safety/toxicity studies in concert with exposure information constitute the two critical information components that form the basis of the GRAS evaluation.

The safety/toxicity studies, consumption/exposure information, and other related documentation were augmented with an independent search of the scientific and regulatory literature, which included two prior GRAS notices filed as GRN No. 209 submitted by Taiyo International, Inc. and GRN No. 338 submitted by Blue California. Based upon the composite information, a GRAS assessment based primarily on available safety information and common occurrence in food was undertaken. Those references that were deemed pertinent to the objective at hand are discussed in the present GRAS dossier.

C. FDA Regulatory Framework

Ingredients for use in foods must undergo pre-market approval by FDA as food additives or, alternatively, the ingredients to be incorporated into foods must be determined to be generally recognized as safe (GRAS). The authority to make GRAS determinations is not restricted to FDA. In fact, GRAS determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process and replacing the petitioning process with a notification procedure. While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

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D. Regulatory History of Theanine

A search of the U.S. FDA inventory of GRAS notices³ for “theanine” revealed two prior documents filed as GRN No. 209 submitted by Taiyo International, Inc. and GRN No. 338 submitted by Blue California. In both cases, FDA indicated based on the available information that it had no questions regarding each company’s conclusion that L-theanine is GRAS under the intended conditions of use, as an ingredient in fruit juices and drinks, non-herbal teas, sports beverages, bottled waters, chocolate bars and chews, hard candies, breath mints, and chewing gum at levels up to 250 milligrams per serving.

The main elements of these two prior GRAS notices, along with those of the present GRAS notice for comparison, are summarized in Table 1 below.

Table 1. Overview of the elements in Tianrui Chemical’s present GRAS notice vs. those of two prior GRAS notices that generated no objections from FDA

	PRESENT GRAS NOTICE	GRN No. 338	GRN No. 209
Company	Zhejiang Tianrui Chemical Co., Ltd.	Blue California	Taiyo International, Inc.
Year	2013	2010	2009
Substance	L-Theanine ($\geq 98\%$)	L-Theanine (L-TeaActive™)	L-Theanine (Suntheanine®)
Source	Chemically-synthesized from L-glutamic acid and ethylamine	Aqueous extraction of tea leaves	Enzymatic (glutaminase from <i>Pseudomonas nitroreducens</i> or <i>Bacillus amyloliquefaciens</i>) synthesis
Produced using GMP?	Yes	Yes	Yes
Specifications	<u>Purity</u> : $\geq 98\%$	<u>Purity</u> : $\geq 98\%$	<u>Purity</u> : $\geq 98\%$
	<u>Specific Rotation</u> : $+7.7^\circ$ to $+8.5^\circ$	<u>Specific Rotation</u> : $+7.2^\circ$ to $+8.5^\circ$	<u>Specific Rotation</u> : $+7.7^\circ$ to $+8.5^\circ$
	<u>pH</u> : 5.0-6.0	<u>pH</u> : 5.0-7.0	<u>pH</u> : 5.0-6.0
	<u>Arsenic</u> : $\leq 4 \mu\text{g/g}$	<u>Arsenic</u> : $< 2 \text{ ppm}$ ($\mu\text{g/g}$)	<u>Arsenic (as As₂O₃)</u> : $< 4 \mu\text{g/g}$
	<u>Heavy Metals</u> : $\leq 10 \text{ ppm}$ ($\mu\text{g/g}$)	<u>Heavy Metals</u> : $< 10 \text{ ppm}$ ($\mu\text{g/g}$)	<u>Heavy Metals (as Pb)</u> : $< 1 \mu\text{g/g}$ (ppm)
	<u>Lead</u> : $\leq 1 \mu\text{g/g}$ (ppm)	<u>Lead</u> : $< 1 \text{ ppm}$ ($\mu\text{g/g}$)	<u>Lead</u> : $< 1 \mu\text{g/g}$ (ppm)
	<u>Total Plate Count</u> : $< 1000 \text{ cfu/g}$	<u>Total Plate Count</u> : $< 3000 \text{ cfu/g}$	<u>Standard Plate Count</u> : $< 3000 \text{ cfu/g}$

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³ GRAS Notice Inventory available online through:

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>.

Table 1. Overview of the elements in Tianrui Chemical's present GRAS notice vs. those of two prior GRAS notices that generated no objections from FDA

	PRESENT GRAS NOTICE	GRN No. 338	GRN No. 209
	<u>Total Coliforms:</u> Negative in 0.1 g	<u>Total Coliforms:</u> < 100 cfu/g	<u>Total Coliforms:</u> Negative in 0.1 g
	<u>Mold/Yeast:</u> < 100 cfu/g	<u>Yeast & Molds:</u> < 100 cfu/g	<u>Mold/Yeast:</u> < 100/g
	<u>Salmonella:</u> Negative	<u>Salmonella:</u> Negative	Not specified
	<u>E. coli:</u> Negative	<u>E. coli:</u> Negative	Not specified
	<u>Shelf-Life:</u> ≥ 24 months	<u>Shelf-Life:</u> 2 years (24 months)	<u>Shelf-Life:</u> ≥ 24 months
Proposed Food Uses	Dietary source of L-theanine in bottled water, chocolate bars and chews, fruit juices and drinks, hard candies/mints, non-herbal teas, sports drinks, chewing gums.	Dietary source of L-theanine in bottled water, chocolate bars and chews, fruit juices and drinks, hard candies/mints, non-herbal teas, sports drinks, chewing gums.	Dietary source of L-theanine in fruit juices, sports beverages, non-herbal teas, bottled water, chocolate power bars and chews, breath mints, hard candies, chewing gum.
Proposed Concentrations	≤ 250 mg/serving	≤ 250 mg/serving	≤ 250 mg/serving
Projected Consumer Exposure (all proposed food categories)	Since the proposed food uses are identical to those in the previous GRAS notices, the projected consumer exposure is expected likewise to be the same.	<u>Mean:</u> 11.3 mg/kg bw/day 628 mg/person/day <u>90th Percentile:</u> 24.2 mg/kg bw/day 1284 mg/person/day [Based on 1999-2002 National Health and Nutrition Examination Survey (NHANES)]	<u>Mean:</u> 11.3 mg/kg bw/day 628 mg/person/day <u>90th Percentile:</u> 24.2 mg/kg bw/day 1284 mg/person/day [Based on 1994-96 Continuing Survey of Food Intakes by Individuals (USDA, 2000)]

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Table 1. Overview of the elements in Tianrui Chemical's present GRAS notice vs. those of two prior GRAS notices that generated no objections from FDA

	PRESENT GRAS NOTICE	GRN No. 338	GRN No. 209
Estimated Background Exposure	Refers to GRN 209 estimates.	Refers to GRN 209 estimates.	<u>Mean:</u> 2.12 to 5.30 mg/kg bw 152.6 to 381 mg/person/day <u>90th Percentile:</u> 4.18 to 10.45 mg/kg bw 330 to 825 mg/person/day [Based on tea leaf-derived beverage with L-theanine content ranging from 1 to 2.5%]

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III. INGREDIENT IDENTITY, CHEMICAL CHARACTERIZATION, MANUFACTURING PROCESS & PURITY

A. Common or Usual Name & Identity of the Notified Substance

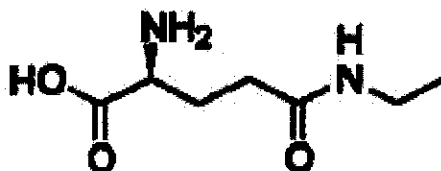
L-Theanine (frequently referred to as theanine) is the common name for the notified substance. The specific material of interest to Zhejiang Tianrui Chemical Co., Ltd. is a high-purity L-theanine, also referred to as *L-theanine* ($\geq 98\%$). The Chemical Abstract Service (CAS) registry number for theanine is 3081-61-6.

B. Chemical Name

The subject ingredient, L-theanine, is an amino acid also known as γ -glutamylethylamide or N-ethyl-L-glutamine.

C. Chemical Identity of Theanine

Figure 1. Chemical Structure of Theanine



L-Theanine has the molecular formula $C_7H_{14}N_2O_3$ and a molecular weight of 174.20 Daltons. Theanine is not incorporated into protein. The chemical structure of theanine is given in Figure 1. A summary of the physical and chemical properties of L-theanine may be found in Table 2.

Table 2. Physical & chemical properties of L-theanine

Appearance:	White crystalline powder
Appearance in solution:	Transparent, colorless
Odor:	None
Taste:	Slightly sweet
Melting point:	214-215°C
Water solubility:	Soluble
Ethanol solubility:	Insoluble
Ether solubility:	Insoluble

D. Manufacturing Process: Biosynthesis

L-Theanine is naturally-occurring at high concentrations in the leaves of the green tea plant, *Camellia sinensis*. It can also be found in the non-edible mushroom *Xerocomus badius*. L-Theanine accounts for approximately 1-2% of the dry weight of tea leaves and is water-soluble (Borzelleca *et al.*, 2006). Steeping *C. sinensis* leaves in hot water creates tea, the most commonly consumed beverage in the world. After steeping, the majority of theanine is solubilized (Ekborg-Ott *et al.*, 1997).

Tianrui Chemical synthesizes *L-theanine* ($\geq 98\%$) chemically from food-grade L-glutamic acid and ethylamine, using purified water and ethanol as solvents. An overview of the manufacturing process used by is depicted in Figure 2. *L-theanine* ($\geq 98\%$) is manufactured in accordance with current Good Manufacturing Practices (cGMP) and its quality is routinely monitored using stringent quality control and quality assurance systems.

Prior to use, the starting materials, L-glutamic acid and ethylamine, are tested for compliance with the specification described in Tables 3 and 4, respectively. The purified water used in manufacturing is obtained from a water circulation system. The reactant is sent to a filtering tank through plate & frame filtration, using active carbon, in order to adsorb impurities, and through the ion exchange resin column to further purify L-theanine solution. The L-theanine solution is concentrated, centrifuged with ethanol to product L-theanine crude product. L-theanine is dried and milled in clean room, and then subjected to metal detection prior to packaging.

Table 3. Specifications for L-glutamic acid (raw material)

Attribute	Specification (A.JI92)
Appearance	White crystals or crystalline powder
Assay	99.0% to 100.5%
Specific rotation(α) ²⁰ D (C=10 in 2N HCL)	+31.5 to + 32.5°
State of solution (Transmittance)	Clear and colorless not less than 98.0%
Chloride(Cl)	$\leq 0.020\%$
Ammonium (NH ₄)	$\leq 0.02\%$
Sulfate (SO ₄)	$\leq 0.020\%$
Iron (Fe)	≤ 10 ppm
Heavy metals (as Pb)	≤ 10 ppm
Arsenic (As ₂ O ₃)	≤ 1 ppm
Other Amino Acids	conforms
Loss on drying	$\leq 0.1\%$
Residue on ignition	$\leq 0.10\%$
pH	3.0 to 3.5

Table 4a. Specifications for ethylamine (raw material) aqueous solution

Attribute	Specification
Appearance	Colorless transparent liquid
Ethylamine	70.0% to 72.0%
Inorganic ammonia	$\leq 0.07\%$
Diethylamine	$\leq 0.07\%$
Triethylamine	$\leq 0.04\%$
Ethanol	$\leq 0.07\%$
Water	$\leq 30.0\%$

Table 4b. Specifications for ethanol (solvent) aqueous solution

Attribute	Specification
Alcoholic(w/w%)	≥ 99.7
Moisture (w/w%)	≤ 0.3
Acidity (mmol/100g)	≤ 0.04
Alkalinity (mmol/100g)	≤ 0.01
Methanol (w/w%)	≤ 0.05
Residue on Evaporation (w/w%)	≤ 0.001
Isopropyl Alcohol (w/w%)	≤ 0.01

E. Product Purity

1. Identification & Purity Analysis of *L-Theanine* ($\geq 98\%$)

To ensure the purified product is chemically identical to *L-theanine*, Zhejiang Tianrui Chemical Co., Ltd. requested that SHANG PHARMA CORPORATION SHANGHAI CHEMPARTNER CO., LTD. (CHEMPARTNER hereafter) perform infrared spectrometry on five lots of purified *L-theanine* ($\geq 98\%$). Overlap of the Fourier transform-infrared spectra of *L-theanine* ($\geq 98\%$) with an USP *L-theanine* reference standard, using FTIR instrumentation, revealed that these two substances are chemically identical. CHEMPARTNER infrared spectrometry results are attached in Appendix A.

Tianrui Chemical also requested that CHEMPARTNER perform purity analyses of five lots of *L-theanine* ($\geq 98\%$) by high-performance liquid chromatography (HPLC). The objective of the study was to determine the purity of the production lots of *L-theanine* powder supplied by Tianrui Chemical. Each sample of the five lots was tested in duplicate and compared to a USP *L-theanine* standard, using the *L-theanine* standard by FCC method, at the 1 mg/mL expected concentration of the samples. The results of the purity analyses are listed in Table 5. Overall, the study confirmed the identity of the purified product as *L-theanine*. The range of purity of *L-theanine* in the sample lots was 99.44%-100.79%, conforming to the specification 98.00%-102.00% in FCC. The detailed

CHEMPARTNER report is attached in Appendix A.

Table 5. Purity analysis results for Tianrui Chemical *L-theanine* ($\geq 98\%$) from five production samples

Lot Number	Assay %
Sample TR20120815	99.44
Sample TR20131015	99.80
Sample TR20121119	100.79
Sample TR20130625	100.75
Sample TR20130925	101.39

i. Enantiomer Content of *L-Theanine* ($\geq 98\%$)

L-Theanine in tea leaves and after solubilization is known to be a mixture of the L-theanine and D-theanine enantiomers. Ekborg-Ott *et al.* (1997) reported that D-theanine constitutes an average of 1.85% of the total theanine in 17 types of tea tested.

To determine the amount of the L-theanine and D-theanine enantiomers in *L-theanine* ($\geq 98\%$), Tianrui Chemical requested that CHEMPARTNER perform a chirality analysis by HPLC on the same five lots from the purity analysis, and to quantify any D-theanine that might be present.

As Table 6 illustrates, the optical rotation analysis (FCC method) performed by CHEMPARTNER confirmed the product as L-theanine. The range of optical rotation of L-theanine in the sample lots was $+7.920^\circ$ to $+8.458^\circ$, conforming to the specification $+7.7^\circ$ to $+8.5^\circ$ in FCC. Duplicate analyses of the same five Tianrui Chemical production lots indicated that D-Theanine was not detected (ND) in any of the samples tested. Therefore, the level of D-theanine present in *L-theanine* ($\geq 98\%$) was determined to be below the LOD (limit of detection).

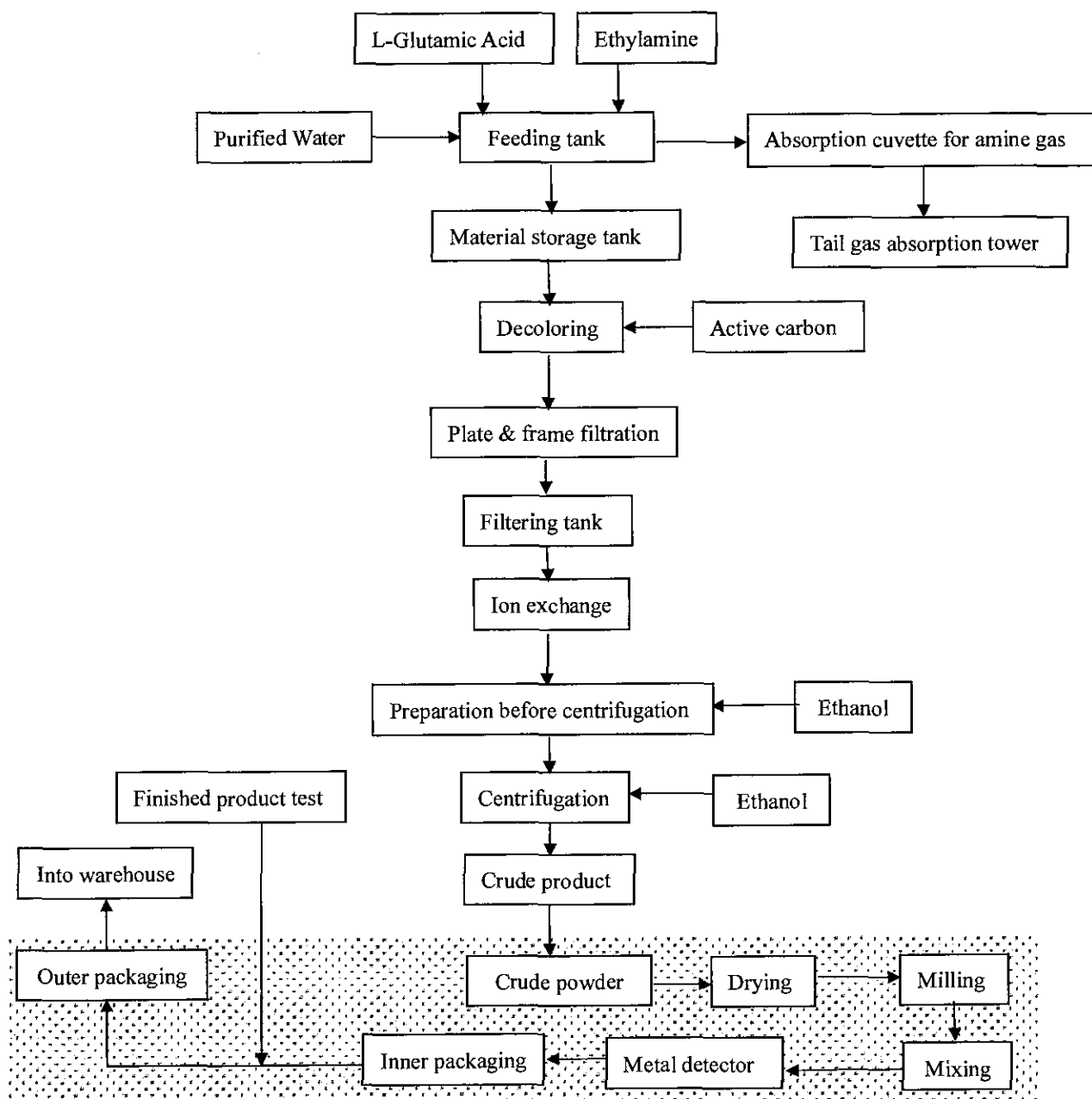
Table 6. Optical rotation for Tianrui Chemical *L-theanine* ($\geq 98\%$) from five production samples

Lot Number	Optical Rotation*
Sample TR20120815	8.458
Sample TR20131015	8.249
Sample TR20121119	7.920
Sample TR20130625	8.199
Sample TR20130925	8.295

Reference Standard (Lot#:LE10103): Optical Rotation=8.259

Figure 2. Manufacturing process for Tianrui Chemical *L-theanine* ($\geq 98\%$)

Process Flow Chart of L-Theanine



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ii. Specifications of *L-Theanine* ($\geq 98\%$)

Zhejiang Tianrui Chemical Co., Ltd. has established specifications for its *L-theanine* ($\geq 98\%$) ingredient, which are intended to maintain the food-grade status of the final product. The food-grade specifications for *L-theanine* ($\geq 98\%$) are listed in Table 7.

Table 7. Food-grade specifications for Tianrui Chemical *L-theanine* ($\geq 98\%$)

Attribute	Specification	Method
Appearance	White crystalline powder	Visual
Taste and Odor	Slight sweet taste with no odor	Sensory test
Solubility (1.0 g/20 ml H ₂ O)	Transparent Colorless	Visual
Assay (dried basis)	98.00%-102.00%	HPLC
Specific rotation(α) ²⁰ D	+7.7° to +8.5°	FCC8
Chloride (Cl)	$\leq 0.021\%$	FCC8
Loss on drying	$\leq 1\%$	FCC8
Residue on ignition	$\leq 0.2\%$	FCC8
pH	5.0-6.0	FCC8
Heavy metals	≤ 10 ppm	GB/T 5009.74
Arsenic	≤ 4 ppm	FCC8
Lead	≤ 1 ppm	FCC8
Total Plate Count	<1000 cfu/g	GB4789.2
Mold and Yeast	<100 cfu/g	GB4789.15
<i>Salmonella</i>	Negative	GB4789.4
<i>E. coli</i>	Negative	GB4789.3

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iii. Analysis of *L-Theanine* ($\geq 98\%$) Production Lots

To demonstrate conformance with the aforementioned established food-grade specifications, Zhejiang Tianrui Chemical Co., Ltd. analyzed five lots of its high-purity *L-theanine*. The results of these analyses can be found in Table 8.

All five lots of *L-theanine* ($\geq 98\%$) were within the established specifications, indicating that the production process is capable of consistently producing food-grade product (see Appendices A and B).

Table 8. Results of analysis of Tianrui Chemical *L-Theanine* ($\geq 98\%$)

Attribute	Specification	Lot Number				
		TR20120815	TR20121119	TR20130625	TR20130925	TR20131015
Appearance	White crystalline powder	PASS	PASS	PASS	PASS	PASS
Taste and Odor	Slight sweet taste with no odor	PASS	PASS	PASS	PASS	PASS
Solubility	Clear Colorless	PASS	PASS	PASS	PASS	PASS
Assay (%)	98.00-102.00	99.44	100.79	100.75	101.39	99.80
Specific rotation	+7.7° to +8.5°	8.458	7.920	8.199	8.295	8.249
Chloride (%)	≤ 0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Loss on drying (%)	≤ 1	0.34	0.13	0.10	0.11	0.15
Residue on ignition (%)	≤ 0.2	0.15	0.08	0.07	0.05	0.11
pH	5.0-6.0	5.48	5.48	5.56	5.46	5.22
Heavy metals (ppm)	≤ 10	<10	<10	<10	<10	<10
Arsenic (ppm)	≤ 4	ND	0.009	0.01	0.01	0.01
Lead (ppm)	≤ 1	0.007	0.010	0.005	0.005	0.005
Total Plate Count (cfu/g)	<1000	<10	<10	<10	<10	<10
Mold and Yeast (cfu/g)	<100	<10	<10	<10	<10	M: 10 Y: <10
<i>Salmonella</i>	Negative	ND	ND	ND	ND	ND
<i>E. coli</i>	Negative	ND	ND	ND	ND	ND

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F. Stability Data

The stability of *L-theanine* ($\geq 98\%$) was tested under various conditions. Table 9 shows the results at room temperature (25°C) and various pH levels over a period of 24 hours. Solutions of *L-theanine* ($\geq 98\%$) (0.2%) were prepared and adjusted by citrate buffer and sodium carboxylate buffer to different pH levels, ranging from pH 2 to pH 10. The theanine assay showed only minimal degradation.

Table 9. Stability of Tianrui Chemical *L-theanine* ($\geq 98\%$) in various pH solutions at 25°C

	0 hours	2 hours	6 hours	10 hours	16 hours	20 hours	24 hours
pH 2	99.85	99.82	99.74	99.62	99.31	99.11	99.11
pH 6	99.63	99.51	98.92	98.82	98.02	97.71	96.80
pH 7	99.37	99.35	98.80	97.40	97.23	97.23	95.98
pH 8	99.29	99.10	99.10	99.03	98.12	97.31	97.01
pH 10	99.41	99.23	99.12	98.82	97.52	96.14	95.22

To further analyze the stability of *L-theanine* ($\geq 98\%$), the same test was conducted at 100°C. As shown in Table 10, under these conditions, the theanine assay showed only slight degradation of *L-theanine* ($\geq 98\%$).

Table 10. Stability of Tianrui Chemical *L-theanine* ($\geq 98\%$) in various pH solutions at 100°C

	0 hours	2 hours	6 hours	10 hours	16 hours	20 hours	24 hours
pH 2	99.85	99.65	99.34	99.33	98.01	97.68	97.17
pH 6	99.63	98.61	98.02	97.14	96.35	96.04	95.25
pH 7	99.37	99.37	98.79	98.65	98.34	98.34	98.26
pH 8	99.29	98.58	98.09	97.92	97.15	96.79	96.09
pH 10	99.41	98.50	98.11	97.86	97.80	97.69	96.50

The stability of *L-theanine* ($\geq 98\%$) under neutral conditions, but at different temperatures, was tested over a 24-hour time period. In these experiments, five different production lot solutions of *L-theanine* ($\geq 98\%$) were prepared and adjusted to pH 6. The samples were held between 100°C and 140°C, and the *L-theanine* content was analyzed at regular time intervals. As shown in Table 11, *L-theanine* ($\geq 98\%$) was essentially unchanged when kept at high temperatures for up to 24 hours.

Table 11. Stability of Tianrui Chemical *L-theanine* ($\geq 98\%$) at pH 6 and different temperatures

	0 hours	2 hours	6 hours	10 hours	16 hours	20 hours	24 hours
100°C	99.85	99.76	99.47	99.30	99.14	99.09	99.01
110°C	99.63	99.63	99.25	98.87	98.81	98.62	98.13
120°C	99.37	99.05	98.99	98.78	98.54	98.23	98.06
130°C	99.29	99.18	99.12	98.56	98.34	98.09	97.98
140°C	99.41	99.25	99.24	99.02	98.74	98.53	98.15

To assess the long-term stability of *L-theanine* ($\geq 98\%$) at room temperature (25°C), two samples were packaged, tightly sealed, and stored for up to 2 years. The *L-theanine* concentration was analyzed at 12 months and 24 months by HPLC. As shown in Table 12, almost no change was seen in the theanine assay and moisture of the samples. This study demonstrates that the shelf-life of *L-theanine* ($\geq 98\%$) at room temperature is at least 24 months.

Table 12. Stability of Tianrui Chemical *L-theanine* ($\geq 98\%$) stored at room temperature

Lot & Months	Assay (%)	Moisture (%)
Lot # 20110109 (2011)	99.48	0.17
After 12 months	- 0.25	+0.04
After 24 months	- 0.33	+0.03
Lot # 20101228 (2010)	99.34	0.24
After 12 months	- 0.11	+0.02
After 24 months	- 0.24	+0.03

IV. INTENDED USE & DIETARY EXPOSURE

Tianrui Chemical intends to use *L-theanine* ($\geq 98\%$) as an amino acid in a limited number of human food categories where the high purity *L-theanine* would function as a nutrient supplement as defined in 21 CFR 170.3(0)(20). The intended foods and use levels, shown in Table 13, are identical to those described in two prior GRAS notices filed by U.S. FDA as GRN No. 209 submitted by Taiyo International, Inc. and GRN No. 338 submitted by Blue California. In both cases, FDA indicated based on the available information that it had no questions regarding each company's conclusion that *L-theanine* is GRAS under the intended conditions of use, as an ingredient in fruit juices and drinks, non-herbal teas, sports beverages, bottled waters, chocolate bars and chews, hard candies, breath mints, and chewing gum at levels up to 250 milligrams per serving.

Table 13. Consumption estimates based on the proposed food uses of *L-theanine* ($\geq 98\%$)

Food category	Serving size (RACC)	Intended addition levels (mg/g)	% User	Daily Intake of L-Theanine			
				Per user (mg/day)		Per kg body weight (mg/kg bw/day)	
				Mean	90 th percentile	Mean	90 th percentile
Bottled Water	240 mL	1.0	<0.1	121	242	8.1	16.2
Chocolate Bars& Chews	50 g	5.0	4.0	181	304	3.8	7.1
Fruit juices & drinks	240 mL	1.0	54.8	385	752	8.2	18.5
Hard Candies/Mints	15 g/2 g	16.7/125	4.4	154	309	3.9	8.4
Non-herbal Teas	240 mL	1.0	30.3	590	1202	8.7	17.0
Sports Drinks	240 mL	1.0	2.2	360	749	6.4	13.2
Chewing Gums	3 g	83.3	50.0	125	250	1.8	3.6
All categories			86.4	628	1284	11.3	24.2

Adapted from GRN No. 209, Table 18.

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Because Tianrui Chemical's *L-theanine* ($\geq 98\%$) ingredient is intended as an alternative to the Taiyo International and Blue California *L-theanine* ingredients already considered GRAS, and the conditions of use are identical, no major impact on the intake of *L-theanine* in the overall diet of the public would be expected. The present case simply constitutes an introduction into the market by

another supplier who will have to compete in essentially the same markets and foods. Table 14 provides a comparison of the three L-theanine ingredients discussed.

Table 14. Comparison of the Taiyo International, Blue California, and Tianrui Chemical L-theanine intended uses and dietary exposure

	PRESENT GRAS NOTICE	GRN No. 338	GRN No. 209
Company	Zhejiang Tianrui Chemical Co., Ltd.	Blue California	Taiyo International, Inc.
Year	2013	2010	2009
Substance	<i>L-Theanine</i> ($\geq 98\%$)	L-Theanine (L-TeaActive™)	L-Theanine (Suntheanine®)
Source	Chemically-synthesized from L-glutamic acid and ethylamine	Aqueous extraction of tea leaves	Enzymatic (glutaminase from <i>Pseudomonas nitroreducens</i> or <i>Bacillus amyloliquefaciens</i>) synthesis
Proposed Food Uses	Dietary source of L-theanine in bottled water, chocolate bars and chews, fruit juices and drinks, hard candies/mints, non-herbal teas, sports drinks, chewing gums.	Dietary source of L-theanine in bottled water, chocolate bars and chews, fruit juices and drinks, hard candies/mints, non-herbal teas, sports drinks, chewing gums.	Dietary source of L-theanine in fruit juices, sports beverages, non-herbal teas, bottled water, chocolate power bars and chews, breath mints, hard candies, chewing gum.
Proposed Concentrations	≤ 250 mg/serving	≤ 250 mg/serving	≤ 250 mg/serving
Projected Consumer Exposure (all proposed food categories)	<p>Since the proposed food uses are identical to those in the previous GRAS notices (GRN No. 209 & GRN No. 338), the projected consumer exposure is expected likewise to be the same.</p> <p><u>Mean:</u> 11.3 mg/kg bw/day 628 mg/person/day</p> <p><u>90th Percentile:</u> 24.2 mg/kg bw/day 1284 mg/person/day</p>	<p><u>Mean:</u> 11.3 mg/kg bw/day 628 mg/person/day</p> <p><u>90th Percentile:</u> 24.2 mg/kg bw/day 1284 mg/person/day</p> <p>[Based on 1999-2002 National Health and Nutrition Examination Survey (NHANES)]</p>	<p><u>Mean:</u> 11.3 mg/kg bw/day 628 mg/person/day</p> <p><u>90th Percentile:</u> 24.2 mg/kg bw/day 1284 mg/person/day</p> <p>[Based on 1994-96 Continuing Survey of Food Intakes by Individuals (USDA, 2000)]</p>

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Table 14. Comparison of the Taiyo International, Blue California, and Tianrui Chemical *L-theanine* intended uses and dietary exposure

	PRESENT GRAS NOTICE	GRN No. 338	GRN No. 209
Estimated Background Exposure	Refers to GRN 209 estimates.	Refers to GRN 209 estimates.	<p><u>Mean:</u> 2.12 to 5.30 mg/kg bw 152.6 to 381 mg/person/day</p> <p><u>90th Percentile:</u> 4.18 to 10.45 mg/kg bw 330 to 825 mg/person/day</p> <p>[Based on tea leaf-derived beverage with <i>L-theanine</i> content ranging from 1 to 2.5%]</p>

Further Details Regarding Dietary Intakes

Based on a statistical analysis of potential dietary intake, it was estimated in GRAS notice GRN 209 that the mean consumption would be 628 mg/person/day, and the 90th percentile consumption would be 1284 mg (1.28 g)/person/day. The dietary analysis below was presented in GRN 209 and was not questioned by FDA in the response letter of February 5, 2007.

Exponent Inc. calculated the daily intake of Suntheanine[®] estimated to result from its intended addition to food. These estimates were based on data collected in the United States Department of Agriculture's 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its Supplemental Children's Survey (CSFII 1998), as provided on CD-ROM (USDA 2000). The CSFII 1994-96 was conducted between January, 1994, and January, 1997, with noninstitutionalized individuals in the United States. Twenty-four-hour diet recall data were collected through two in-person interviews conducted between 3 and 10 days apart from a nationally representative sample of individuals of all ages. The CSFII 1998 was designed as a supplement to CSFII 1994-96 to increase the sample size for children from birth through age 9 years. This survey employed the same methodology as CSFII 1994-96. In the merged surveys (designated CSFII 1994-96, 1998), 21662 individuals provided dietary intake data on the first survey day, and 20607 provided data on the second day. The CSFII was selected, rather than the more recent NHANES 1999-2002, because the availability of 2-day data allows better estimation of the usual intake of Suntheanine[®] potentially resulting from its intended use.

EDIs are calculated by multiplying the reported consumption of each food in categories in which Suntheanine[®] addition is intended by the maximum intended addition level as shown in Table 17. The total EDI is calculated by adding, at the level of the individual respondent, the intakes of Suntheanine[®] from each use in foods consumed by the individual. The results of these calculations are shown in Table 18.

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Of the food categories in which Suntheanine® is intended to be used, fruit juices and drinks and chewing gum are by far the most widely consumed, by 50% or more of the United States population. Over 85% of the respondents to the CSFII reported consuming at least one of the target foods on at least one of the two survey days. On average, it is anticipated that individuals will consume 628 mg of Suntheanine® per day (11.3 mg/kg bw/day). The EDI of Suntheanine® under its intended conditions of use corresponds with the estimated 90th percentile of intake, or 1284 mg (24.2 mg/kg bw). These are roughly similar to the levels of L-theanine currently consumed by the heaviest tea drinkers in the United States, as was shown in Table 16.

Further Details Regarding Background Exposure

The only naturally-occurring source of theanine in the American diet is tea. Behind water, tea is the most widely consumed beverage in the world. Approximately 20% of Americans consume tea each day. This percentage is higher in many other countries. In the 1999-2002 National Health and Nutrition Examination Survey (NHANES), the mean amount of tea consumed by an individual was 763 mL, but the top 10% of consumers drank 1650 mL of tea and the top 1% of consumers drank 3337 mL.

Approximately 1-2% of the dry weight of tea leaves is theanine and the majority of theanine is solubilized when tea is prepared. Recent studies by Kato *et al.* (2003) and Ekborg-Ott *et al.* (1997) found the average theanine concentration among tea varieties was approximately 1.4%. A typical cup of tea uses 3 g of tea leaves, which contains about 30 mg of theanine. Based on the typical concentration of L-theanine in tea (1-2.5%), in GRN 209 (2006) the estimated daily intake of L-theanine from tea was determined to be between 153 and 382 mg/person/day at the mean and between 330 and 825 mg/person/day at the 90th percentile. These estimates indicate that the estimated daily intake of L-theanine under the proposed intended conditions of use of L-theanine ($\geq 98\%$) corresponds with the levels of L-theanine currently consumed by the heaviest tea drinkers in the United States.

In summary, the intended uses of L-theanine ($\geq 98\%$) will result in an estimated 90th percentile daily intake of 1284 mg. The background 90th percentile intake of L-theanine from its presence in tea has been estimated as 825 mg/day. The estimated daily intake is roughly similar to the levels of L-theanine currently consumed by the heaviest tea drinkers in the United States.

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V. REVIEW OF THEANINE SAFETY DATA

As already mentioned, the proposed conditions of use of Tianrui Chemical's *L-theanine* ($\geq 98\%$) ingredient are identical to those described for similar L-theanine ingredients in GRAS notices GRN Nos. 209 (Taiyo International, Inc.) and 338 (Blue California), which generated no questions from FDA. As such, the information supporting the safety of L-theanine in these two GRAS notices would likewise be expected to support the safety of Tianrui Chemical's *L-theanine* ($\geq 98\%$) ingredient. This information is repeated herein.

A. Common Knowledge of Safe Theanine Consumption

In the form of tea, theanine is a widely consumed ingredient that has been a part of the human diet for thousands of years. After water, tea is one of the most widely consumed beverages in the world (Graham 1992; Blumberg 2003). World tea production in 2001 was reported to be 3.02 million tons. Although the United States is not considered a major tea-consuming country, over 20% of NHANES survey respondents age 13 and older and nearly 20% of respondents of all ages reported having consumed tea on the survey day. Their average intake of tea, on days they consumed tea, was nearly 800 g or about 28 ounces. The average worldwide per capita daily intake of tea has been estimated to be about 120 mL (Blumberg, 2003). Because this per capita intake includes infants and toddlers, as well as older non-tea drinkers, it is evident that consumption among older children, adolescents, and adults is much higher. Based on a 2003 world population of about 6.2 billion, per capita consumption of 120 mL of tea equates to daily consumption of nearly a million metric tons of tea. Thus, the available evidence suggests that there is a common exposure to tea and to its constituent, L-theanine. Despite this long history, no adverse effects due to ingestion of tea or L-theanine have been reported. L-Theanine has also been used as a dietary supplement in the US since the 1990s without any reports of adverse effects.

B. Absorption, Distribution, Metabolism & Excretion of Theanine

Because data on the metabolic fate of L-theanine from human studies are lacking, Lisa Scheid *et al.* (2012) investigated the kinetics of L-theanine uptake and its metabolites, ethylamine and glutamic acid, in healthy participants. Within a randomized crossover study, 12 participants ingested a bolus of 100 mg L-theanine *via* capsules or green tea. On further occasions, 3 participants received 50 and 200 mg L-theanine *via* capsules. Blood and urine were collected before and up to 24 h post consumption to determine the concentrations of L-theanine, proteinogenic amino acids, and ethylamine in plasma, erythrocytes, and urine by HPLC. L-Theanine increased in plasma, erythrocytes, and urine with comparable results after both treatments. The maximum plasma concentration of L-theanine occurred 0.8 h after intake of 100 mg L-theanine *via* capsules (24.3 ± 5.7 $\mu\text{mol/L}$) and tea (26.5 ± 5.2 $\mu\text{mol/L}$), respectively. The AUC of L-theanine in plasma increased in a dose-dependent manner after intake of 50, 100, and 200 mg L-theanine *via* capsules. Moreover, ethylamine and glutamic acid increased in plasma and were excreted by urine after intake of capsules and tea.

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In conclusion, L-theanine is rapidly absorbed and seems to be hydrolyzed to ethylamine and glutamic acid. A minor part of L-theanine is retained in erythrocytes. Kinetics and urinary excretion of L-theanine, ethylamine, and glutamic acid are comparable after both treatments.

After consumption of tea, L-theanine enters the systemic circulation and is assumed to enter the brain. Several human studies indicate that L-theanine influences brain functioning. Knowledge about the pharmacokinetics of L-theanine facilitates further study of this health effect (PC Van Der Pijl, 2010). Volunteers received 25–100 mg of L-theanine as tea, as L-theanine-enriched tea, and as biosynthetic L-theanine in aqueous solutions. Plasma was analyzed for L-theanine content after which data were fitted with a 1-compartment model. For all interventions, the lag time was approximately 10 min and half-lives of absorption and elimination were approximately 15 and 65 min, respectively. After approximately 50 min, maximum plasma concentrations of between 1.0 and 4.4 mg/L were achieved. Maximum plasma concentration and area under the plasma-concentration–time curve were dose-proportional. This knowledge allows prediction of plasma concentrations for various dose regimens supporting further study of a health benefit of L-theanine.

L-Theanine obtained from various sources (pure, biosynthetic, and tea-derived) is quickly absorbed and eliminated from the systemic circulation. The pharmacokinetic behavior of L-theanine, independent of source, is very similar for simple matrices like water and tea. The pharmacokinetic model can be used to correlate plasma kinetics within the average tea-consumption dose range with potential benefits of L-theanine. The model will facilitate the design of studies related to L-theanine effects (P.C. Van Der Pijl, 2010).

After consumption in the form of green tea, L-theanine is absorbed in the intestine and distributed throughout the body. Kitaoka *et al.* (1996) studied absorption of L-theanine in guinea pig ileum. Their results suggest that a sodium-coupled electrogenic transporter in the brush border of the intestine mediates absorption of L-theanine. This is the major transport system for dipolar amino acids. Kitaoka *et al.* (1996) found that the sodium-coupled transporter has a 7-fold lower affinity for theanine when compared to glutamine, based on the K_m value.

Following ingestion, L-theanine distributes rapidly throughout the body. Terashima *et al.* (1999) measured the concentration of theanine in the serum, liver, brain, and urine of rats following intragastric administration (4g/kg bw). The concentrations of theanine in the serum, liver, and brain were significantly increased one hour after intragastric administration. Maximum levels in the serum and liver were reached after one hour, while the maximum level of theanine in the brain was reached after 5 hours. Similarly, Unno *et al.* (1999) found that plasma theanine levels peak at 0.5 hours after ingestion. Yokogoshi *et al.* (1998b) found a dose-related increase in theanine levels in rat brain at 2 hours after intragastric administration of theanine (0, 1000, 2000, 4000 mg/kg bw). At the same time, a high dose of theanine led to a significant decrease in the large neutral amino acids (phenylalanine, tryptophan, threonine, tyrosine, and branched-chain amino acids) in rat brain. The levels of other amino acids were unchanged. From these data, the authors concluded that theanine is transported across the blood-brain barrier by the same leucine-preferring transport system utilized by the large, neutral amino acids.

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L-Theanine does not accumulate in the plasma, but is hydrolyzed mostly in the kidney and then excreted from the body. Tsuge *et al.* (2003) examined 10 major tissues from Wistar rats, and detected theanine-degrading enzymatic activity in the kidney homogenate. This activity was not identified in any other tissue homogenates. Later studies indicate that some metabolism of theanine may also occur in other normal tissues, most notably the liver, mediated by glutaminase and γ -GTP (Sugiyama *et al.*, 2004, Sadzuka *et al.*, 2006). In an *in vitro* experiment, Tsuge *et al.* (2003) purified the theanine-degrading enzymatic activity from rat kidney and found it to be consistent with the previously-described phosphate-independent glutaminase. This enzyme also possesses a γ -glutamyl transpeptidase activity. The phosphate-independent glutaminase degrades theanine into glutamic acid and ethylamine *via* a γ -glutamyl transpeptidase reaction. The authors state that some amount of ethylamine may recirculate in the blood, but the majority is excreted in the urine. Concentrations of glutamine and ethylamine increase in urine following theanine administration to rats in a dose-dependent manner (Unno *et al.*, 1999). Theanine completely disappears from all tissues 24 hours after its administration (Terashima *et al.*, 1999).

In a toxicokinetic adjunct to a subchronic study (see Section V.D.3 for additional details of this study) in rats, Borzelleca *et al.* (2006) found a dose-related increase in theanine plasma levels. In this study, blood samples for toxicokinetics were collected at 18:00 hour on Day 17 and at 00:00, 03:00, 06:00, 12:00, and 18:00 hours on Day 18. Blood was also collected at 18:00 hour on Day 85 and at 00:00, 03:00, 06:00, 12:00, and 18:00 hours on Day 86. The increases in C_{\max} and AUC_{0-t} values were approximately proportional to the increase in the dose level during both collection intervals. For most groups, the T_{\max} fell during the dark cycle, consistent with the eating pattern of rodents. Plasma concentrations, C_{\max} and AUC_{0-t} were similar from week 3 to week 13, indicating theanine did not accumulate over time with repeated oral exposure.

C. Biologic Activity of Theanine

L-Theanine, an amino acid in green tea, is suggested to improve cognition and mood. Therefore, L-theanine is available as a supplement and is now used as an ingredient in functional drinks.

Since L-theanine crosses the blood-brain barrier, and is an analog of the major excitatory neurotransmitter glutamate, a number of studies have examined the effects of L-theanine on neural tissues. Research has shown that L-theanine is capable of binding to both glutamate receptors and glutamate transporters. Glutamate receptors are mostly found in the post-synaptic cells, and are responsible for transmitting the excitatory glutamate signal. The glutamate transporters are mostly found in neuronal and glial cells; they remove glutamate from the extracellular synaptic space and help to regulate glutamate levels and thus neuronal signaling. In brain injury and disease, an excess of glutamate causes oxidative damage, known as excitotoxicity, which can ultimately lead to neuronal cell death. Theanine appears to provide neuroprotection by reducing levels of glutamate, thus inhibiting glutamate-induced excitotoxicity. A summary of these studies is found below.

Research studies have shown that theanine binds to both the ionotropic and metabotropic glutamate receptors. The ionotropic glutamate receptors conduct neuronal signals through ligand-gated ion channels. In an early study, Shinozaki and Ishida (1978) reported that theanine functions as a

glutamate antagonist at the crayfish neuromuscular junction. Later, Kakuda *et al.* (2000) demonstrated that theanine provided dose-dependent protection from ischemic delayed neuronal death induced by glutamic acid in field CAI of the gerbil hippocampus. Following this study, Kakuda *et al.* (2002) reported that theanine binds to all three ionotropic glutamate receptors--the NMDA receptor, the kainite receptor, and the AMPA receptor--but with an IC_{50} of theanine that is 80- to 30,000-fold less than that of L-glutamic acid. Their results suggest that theanine acts on the glutamate receptors as an antagonist, but the binding activity is very mild.

Other studies have examined the effects of theanine on the metabotropic glutamate receptors (mGluR). The metabotropic glutamate receptors conduct neuronal signals through a G-protein signaling cascade. Using primary cultured rat neurons, Nagasawa *et al.* (2004) reported that theanine inhibited the delayed death of neurons caused by a brief exposure to glutamate. This effect was abolished by group I mGluR antagonists. Nagasawa *et al.* (2004) also reported that theanine restored the glutamate-induced decrease in protein expression levels of PLC- $\beta 1$ and $\gamma 1$. Again, this effect was abolished by group I mGluR antagonists. Nishida *et al.* (2008) continued these studies *in vivo* (also discussed in section V.D.2). Following oral administration of theanine [10 mmol (1.74g)/kg/day] to rats *via* gastric intubation, the authors report that protein expression of PLC- $\beta 1$ and $\gamma 1$ were significantly increased in the cerebral cortex. A non-significant increase was also reported in the cerebellum, and no change was observed in the hippocampus. The authors conclude that repeated oral administration of theanine reduces oxidation levels in the brain, especially the cortex, suggesting a favorable effect on the brain.

Early studies indicated that theanine also binds to glutamate transporters. Sugiyama *et al.* (2001) reported that theanine significantly inhibited the uptake of glutamate, in a concentration- dependent manner, by M5076 ovarian sarcoma cells. RT-PCR and Western blots revealed that M5076 cells expressed the GLAST and GLT-1 glutamate transporters. The authors suggest that theanine competitively inhibits glutamate uptake by acting on these transporters. However, in a recent study, Kakuda *et al.* (2008) reports that the chemical structure of theanine is more similar to L-glutamine than to glutamate with regard to the amide moiety. In support of this, L-theanine is degraded by phosphate-independent glutaminase (like L-glutamine) and not by phosphate- dependent glutaminase (Tsuge *et al.*, 2003). The authors report the saturable accumulation of [3H]theanine, in a temperature- and sodium-dependent manner, in rat brain synaptosomal fractions and cultured rat cortical neurons. Based on these results, the authors suggest that theanine modulates the glutamate/glutamine cycle in neurons by inhibiting different glutamine transporters.

L-Theanine has also been linked to changes in other neurotransmitters, including serotonin, dopamine, and GABA. Yokogoshi *et al.* (1998a) reported an increase in brain tryptophan and a decrease in brain serotonin in rats following oral L-theanine administration. Microinjection of theanine into rat brain has been reported to increase in glycine and dopamine release in the striatum (Yokogoshi *et al.*, 1998; Yamada *et al.*, 2009). The neuroprotective effect of theanine may also be mediated by GABA(A) receptors. Egashira *et al.* (2004) reported that theanine (1 mg/kg) reduced the size of cerebral infarcts and expression levels of NeuN, GFAP, and Iba 1 at 24 hours after a 4-hour middle cerebral artery occlusion in mice. This effect was inhibited by bicuculline

(GABA(A)-receptor antagonist, 10 mg/kg) but not 3-mercaptopropionic acid (glutamate decarboxylase inhibitor).

Theanine has been investigated for its potential use as neuroprotectant, against Parkinson's Disease and Alzheimer's Disease. Cho *et al.* (2008) reported that L-theanine protects neurons against apoptosis induced by the Parkinson's Disease (PD)-related neurotoxins rotenone and diethyldithiocarbamate. Kim *et al.* (2009) recently reported that L-theanine (2 mg and 4 mg/kg), orally administered to mice in drinking water, attenuated AP(1-42)-induced neuronal cell death (a major pathological mechanism of Alzheimer's Disease) in the cortex and hippocampus of the brain.

In addition to its potential neuroprotectant effects, theanine may also act as a relaxant, as an immune system booster, and as an adjuvant chemotherapeutic agent. In addition, L-theanine may boost concentration and decrease blood pressure. Kobayashi *et al.* (1998) administered either 50 mg or 200 mg theanine in water to four high- and four low-anxiety subjects. In these subjects, an increase in the production of alpha waves (characteristic of relaxation) on electroencephalogram (EEG), but not theta waves (indicators of drowsiness) were observed beginning 30-40 minutes after administration. In a randomized placebo-controlled double-blind crossover study, Song *et al.* (2002) also administered 200 mg L-theanine every day for 7 days to 20 subjects classified as either high- or low-anxiety. Subjects were evaluated 1 hour after administration using the Fatigue Severity Scale and EEG. Both endpoints showed significant relaxant effects of theanine.

As summarized in GRN 209 (2006), Nozawa *et al.* (1995) studied the effects of theanine on brain function *in vitro* using cultured cortical neurons. The addition of theanine was found to increase the calcium in the neurons. The authors concluded that their findings suggest that theanine is a glutamate analog that is stimulatory at low concentration, but a sedative at high concentration and can suppress the excitation produced by caffeine.

L-theanine has also been implicated as an immune system booster. Kurihara *et al.* (2007) report that co-administration of L-cystine and L-theanine to mice, prior to immunization, enhanced antigen-specific IgG production. In humans, Miyagawa *et al.* (2009) found that co-administration of L-cystine and L-theanine (p.o. for 14 days) to elderly patients (n=32) prior to influenza vaccination enhanced the immune response in patients with low serum total protein or hemoglobin when compared to placebo (n=33). In addition, results by Murakami *et al.* (2009), in a randomized, double-blind, placebo-controlled study with 15 long-distance runners, suggest that oral supplementation with L-cystine and L-theanine prevented a decrease in immune function associated with intense training.

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L-Theanine may exert positive effects in other conditions of stress. Studies have shown that theanine may be effective against alcoholic liver injury (Sadzuka *et al.*, 2005), retinopathy of prematurity (Hiramatsu *et al.*, 2008), and may also be useful for reducing raised blood pressure (Yokogoshi *et al.*, 1995; Rogers *et al.*, 2008). The anti-oxidative stress activity of L-theanine may protect normal cells from some of the adverse effects of chemotherapy treatment. Available studies suggest that theanine inhibition of the glutamate transporters leads to the inhibition of efflux of chemotherapeutic agents from tumor cells, enhancing the anti-tumor effects of these agents (Sugiyama and Sadzuka, 2001;

Sadzuka *et al.*, 2002a,b; Sugiyama and Sadzuka, 2003). Recently, Liu *et al.* (2009) found that theanine suppressed the *in vitro* and *ex vivo* growth of human non-small cell cancer A549 and leukemia K562 cell lines in dose- and time-dependent manners. In addition, theanine enhanced the anti-cancer activity of the anti-tumor agents such as trichostatin A, berbamine, and norcantharidin. As a result of these studies, L-theanine has been implicated as a potentially important adjuvant to chemotherapy.

In summary, theanine appears to reduce glutamate-induced excitotoxicity and thus protects neurons from oxidative damage. Its neuroprotective effects may be mediated by binding to glutamate receptors, transporters, or by modulating the glutamate/glutamine cycle. In addition to its neuroprotectant effects, theanine may also act as a relaxant, as an immune system booster, and as an adjuvant chemotherapeutic agent. It may boost concentration and decrease blood pressure.

D. Toxicology Studies on Theanine

Toxicological studies of L-theanine are composed of acute, subacute, subchronic, and chronic toxicity studies in rats and in mice, in addition to *in vitro* assays for mutagenicity and genotoxicity.

The edible safety of theanine was investigated according to Standard of Health Food Test and Assessment (TAN Jun-Feng *et al.*, 2011). Acute toxicity testing in mice by TAN Jun-Feng *et al.* (2011) showed that the maximum tolerance dose (MTD) was greater than 20 g/kg. All three genetic tests including Ames test, mouse bone marrow micronucleus test, and mouse teratospermia test showed negative results. Thirty-day feeding test showed that physical appearance and the indices of hematology, biochemistry and organ coefficients of rats had no significant difference when compared with those in the control group. All these results suggest that compound theanine preparation is highly safe with neither acute toxicity nor genotoxicity.

1. Acute Studies

As reported in GRN 209, Wistar rats were given 2500 or 5000 mg of Suntheanine®/kg bw dissolved in 0.1 mL saline/10 g bw, or 0.1 mL saline/10 g bw alone (control). Rats were observed for 7 days, and body weights were measured. One high-dose female rat died due to an accidental dislocation of the neck. No other effects were noted, and no statistically significant differences in body weights were observed between the control and treatment groups. These data suggest the oral LD₅₀ of Suntheanine® to be >5000 mg/kg bw.

In another acute study, Sadzuka *et al.* (2006) administered L-theanine to male CDF mice (100 mg/kg bw/day, i.p.) for five days in the presence and absence of doxorubicin (DOX). Toxicological analysis was limited to the six cytochrome P450 enzymes (CYP1A1, CYP3A, CYP1A2, CYP2B, CYP2C9, CYP2E1, CYP3A) and glutathione S-transferase (GST) in the liver. The authors found no significant change in the cytochrome P450 content of the liver of mice treated with theanine alone. They also found that, with theanine treatment alone, there was no significant change in the level of activity of any of the CYP subtypes studied or in the level of hepatic GST activity. These results contrasted with the effects of DOX, which decreased the cytochrome P450 content of the liver by 45%, had a variety

of effects on the six cytochrome enzymes, and increased hepatic GST activity. The authors conclude that since theanine did not change CYP activity, it is safe as a food or supplement.

2. Subacute Studies

In one subacute theanine toxicity study, Nishida *et al.* (2008) orally administered L-theanine [10 mmol (1.74 g)/kg, once a day] to rats *via* gastric intubation for 2 weeks. Toxicological evaluation was limited to examining brain and body weights. Despite the administration of a high dose of theanine, equivalent to 10,000 or more cups of green tea, no change was observed in the weight of the body or the cerebral cortex (Cx), cerebellum (Cb), or hippocampus (Hip) in the brain. The authors conclude that 2-week repeated administration of theanine did not have any effect on body weight or on the 3 brain regions studied. Overall, the authors declare theanine to be a safe compound.

A second subacute study of L-theanine, performed by the Nippon Bio Research Center Co., was described in GRN 209. In this study, CRj:CD (SD) rats were gavaged with 2000 mg/kg bw/day of Suntheanine[®] for 28 days. The rats were monitored for changes in body weight, feeding pattern, ophthalmological examination, urine composition (color, occult blood, pH, protein, sugar, ketone bodies, urobilinogen, and bilirubin), hematology (red blood cells, hemoglobin, hematocrit, platelet, white blood cells, mean corpuscular volume, mean concentration of hemoglobin, percent white blood cells, and reticulocyte count), serum clinical chemistry (aspartate aminotransferase, alanine aminotransferase, total cholesterol, triglycerides, total protein, urea nitrogen, creatinine, total glucose, inorganic phosphorus, calcium, sodium, and potassium), and gross and histopathological examination of all of the major body organs.

The authors reported no adverse clinical signs, and no differences between treatment and control groups for body weight, feed consumption, ophthalmological examination, and urinalysis. In the hematologic and biochemical parameters measured, significant changes among treated male rats *vs.* controls included higher fibrinogen (250.8 \pm 5.2 *vs.* 271 \pm 9.0 mg/dL), lower platelets (118.02 \pm 6.23 $\times 10^4/\text{mm}^3$ *vs.* 108.62 \pm 2.29 $\times 10^4/\text{mm}^3$), higher alkaline phosphatase (186.34 \pm 74.51 IU/L *vs.* 283.00 \pm 48.10 IU/L) and greater b-globulin in the protein fraction (11.10 \pm 0.48% *vs.* 11.94 \pm 0.59%). Upon examination of the organs, the authors found no gross pathological changes. Absolute brain weights (1.998 \pm 0.041 g *vs.* 1.884 \pm 0.072 g), but not relative brain weights, were reduced among treated males. In addition, they observed an increase in absolute and relative liver weights. Upon histopathological examination of treated male rats, the authors reported finding slight myocardial denaturation in the hearts of two rats, slight lymphocyte infiltration in the stroma of the kidney and prostate of one rat. In treated females, the authors found one rat with a slight heterotopia thyroid, one had a slight mineral deposition in the stomach, and a third rat had a vestigial postbrachial body in the thyroid. The authors concluded that these findings were likely spontaneous and do not constitute evidence of toxicity. A no-observable-adverse-effect level (NOAEL) of 2000 mg/kg bw/day was proposed.

3. Subchronic Studies

Borzelleca *et al.* (2006) performed a 90-day subchronic study specifically aimed at investigating the safety of L-theanine. The study, which measured the toxicity and toxicokinetic parameters of L-theanine in Sprague-Dawley, was conducted according to OECD and FDA guidelines.

Overall, the authors reported that the oral administration of L-theanine at doses of 1500, 3000, and 4000 mg/kg bw/day resulted in a NOAEL up to the highest dose tested. The authors reported no statistically significant treatment-related adverse effects on morbidity and mortality, body weight, food consumption and efficiency, clinical chemistry, hematology, or urinalysis. Some effects observed by the authors, but not thought to be treatment-related, include a significant decrease in both body weight and food consumption at the 3000 and 4000 mg/kg dose. This decrease was considered to be related to the unpalatability of the diet and was not a toxic response. The authors also observed a decrease in locomotor activity in males when tested at week 3 (3000 mg/kg and 5000 mg/kg) and week 14 (1500 mg/kg, 3000 mg/kg and 4000 mg/kg). The biological significance of this effect in males is unknown, but the authors concluded that this was not a treatment-related adverse effect.

No consistent treatment-related adverse effects on gross pathology, organ weights or ratios, or histopathology were observed in this study. The few effects observed were not consistent over time and/or were not dose-related. The only effects observed by the authors that were thought to be treatment-related were 1) mildly higher serum cholesterol levels in females (3000 mg/kg and 4000 mg/kg), 2) mildly lower serum total protein in females (4000 mg/kg), and 3) mildly higher urine pH in males (3000 mg/kg and 4000 mg/kg). The authors stated that these findings were mild, were not statistically significant, and are not considered adverse effects. Numerous scattered significant alterations in organ weight were observed in the groups at the two sacrifice periods. Significant changes included increased kidney-to-body weight ratios in males at all doses and females at two dose/sacrifice points. Reduced thymus mean weights and thymus-to-body ratio, and thymus-to-brain ratio were also observed in males at all doses. The authors attributed these changes to the overall change in body weights in the treated groups. No histological changes were observed in these organs, and the authors determined that the observed changes were not biologically important.

Borzelleca *et al.* (2006) observed multiple renal tubular cell adenomas and an atypical tubular hyperplasia in 2/20 (10%) of the high-dose (4000 mg/kg) and 1/20 (5%) of the mid-dose (3000 mg/kg) female rats. Except for these three females (two in the high- and one in the mid-dose group), no other females or males showed any evidence of hyperplasia or renal tumors at any of the doses. Toxic tubular changes were not seen in any other animals as would be expected in a direct toxic reaction. Hall *et al.* (2007) extensively investigated the mechanism behind the development of these adenomas and hyperplasia. Their investigation suggests that the increased incidence of renal tubular cell adenomas in high-dose females is due to a genetic predisposition as opposed to direct organ toxicity. The two high-dose affected females were found to be genetically related based on microsatellite DNA loci testing. Hall *et al.* (2007) found that the two high-dose affected females

shared an uncommon polymorphism in the Birt-Hogg- Dube (BhD) gene. Defects in the tumor suppressor gene BhD have been previously linked to a predisposition to spontaneous renal tumors in Sprague-Dawley rats (Kouchi *et al.*, 2006). Supporting a genetic predisposition to renal tumors in the affected rats, Hall *et al.* (2007) performed laser capture microdissection (LCM) of the tumor cells from the affected animals and found a loss of heterozygosity of this gene in one of the two animals. Overall, results from these investigations suggest that the tumors noted in these animals were spontaneous as a result of a shared genetic susceptibility resulting in mutation of the Bhd gene and development of renal tumors. The authors concluded that the development of adenomas and hyperplasia were not related to the test article (Hall *et al.*, 2007).

Fujii and Inai (2008) reported a subchronic and a chronic study of L-theanine in mice. These studies were also summarized in GRN 209 with some additional details. For the subchronic study, B6C3F1 mice (10/sex/group) were fed a diet containing 0, 0.6, 1.25, 2.5 or 5% L-theanine. The approximate daily intake of theanine for male mice was reported as 0, 850, 1700, 3150, and 6300 mg/kg bw/day and for females the intake was 0, 550, 1450, 2550 and 5150 mg/kg bw/day, respectively. Body weight and feed consumption were recorded weekly. At the end of the 13 week test period, all animals were euthanized and autopsied. Organs and tissues, including heart, lung, submaxillary gland, esophagus, stomach, small intestine, large intestine, liver, pancreas, kidney, ovary, thyroid gland, adrenal gland, thymus, spleen, bone marrow, brain (cerebrum, cerebellum) and spinal cord were processed for histological examinations. No mortality or statistically significant differences in body weights and feed consumptions among the test groups were noted. Pathological examinations did not reveal any notable changes, including degeneration, atrophy, necrosis, inflammation or tumor in any organs or tissues. The results of this study suggest that L-theanine had no adverse effects at dose levels of up to 6300 mg/kg bw/day in male and 5150 mg/kg bw/day in female mice.

4. Chronic Studies

In a chronic toxicity study of L-theanine in mice, Fujii and Inai (2008) orally administered L-theanine at dose levels of 0 (control), 2.5 and 5% in the diet to three groups of B6C3F1 mice for 78 weeks. Some additional details of the study were also summarized in GRN 209. Based on the summary provided in the GRN 209, the doses of L-theanine were approximately 0, 2200 and 4200 mg/kg bw/day for males and 0, 2000 and 4150 mg/kg bw/day for females. Compared to control group, no significant change in the mean body weight of mice or in the survival rates in the test group were noted. Over the course of the 78 weeks, the total number of tumors decreased in a dose-dependent manner in both sexes of mice. A statistically significant decrease in the total number of tumors was observed in male mice. A non-significant decrease in tumor number was also observed in female mice.

Given the lack of effect of theanine administration on body weight and organ histology, the authors concluded that the maximum tolerated dose (MTD) is greater than 5%. The investigators concluded that middle to long-term daily administration of L-theanine to mice would cause no undesirable effects.

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5. Genotoxicity/Mutagenicity Studies

Ames tests of L-theanine have been reported by two different groups of investigators. Both groups found L-theanine to be non-mutagenic. Ishidate *et al.* (1984) performed Ames testing and chromosomal aberration tests of 242 different food additives, including L-theanine. The authors reported that an Ames test of L-theanine using *Salmonella typhimurium* and a maximum dose of 20 mg/plate of L-theanine was negative. Fourteen of the 242 food additives tested were positive. As reported in GRN 209, the Nippon Bio Research Center Co. (1999) also performed an Ames test of L-theanine using *S. typhimurium* TA100, TA98, TA1535, and TA1537 and *E coli* strains WP2uvrA with a maximum dose of 5000 $\mu\text{g}/\text{plate}$ of Suntheanine[®] with and without activation (S9). Suntheanine[®] was not mutagenic in this assay.

Similarly, two groups have reported the results of chromosomal aberration tests. As part of the study cited above, Ishidate *et al.* (1984) treated the Chinese hamster fibroblast (CHL) cell line with 242 different food additives, including L-theanine at a maximum dose of 2 mg/mL. Cells were treated at 24 and 48 hours without metabolic activation. Chromosomes were isolated, and a hundred well-spread metaphases were observed under the microscope. The authors reported an incidence of 1 % polyploid cells after 48 hours of L-theanine treatment, and of 4% structural chromosomal aberrations after 24 hours of L-theanine treatment. Results were considered negative if the incidence of aberrations was less than 4.9%. Untreated and solvent treated negative controls had an incidence of 3%. The results of this study suggest that L-theanine does not induce chromosomal aberrations.

In GRN 209 (2006), findings from an unpublished *in vitro* cytogenetics assay by Lloyd (2004) using duplicate human lymphocyte cultures under GLP and following OECD guideline 473 were described. The author reported that the frequency of cells with structural aberrations was similar between L-theanine-treated cultures and the negative sterile purified water controls. In contrast, the positive controls of mitomycin C (without S9) and cyclophosphamide (with S9) demonstrated a significant increase in the proportion of cells with structural aberrations. The authors concluded that L-theanine does not induce chromosome aberrations in human peripheral blood lymphocytes with or without metabolic activation.

E. Clinical Studies on Theanine

Clinical studies of theanine have been performed with theanine administered at dose ranges from 50-250 mg. The majority of human studies performed were with a small sample size and a short duration of exposure. These studies were designed to evaluate the effects of theanine on cognition, attention and stress responses. In all of the studies, limited monitoring was performed for the occurrence of any adverse effects. The results of these studies are summarized below.

A number of clinical studies have utilized ECG recordings to measure changes in alpha band activity in response to L-theanine. Alpha bands are generally thought to be a measure of relaxation. Juneja *et*

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al. (1999) found that oral administration of theanine (50 mg or 200 mg) in four high-anxiety and four low-anxiety volunteers resulted in an increase in alpha waves in the occipital and parietal regions of the brain when compared to water ingestion. Changes in alpha band activity may also be associated with selective attentional processes. Gomez-Ramirez *et al.* (2007) measured alpha band activity in fifteen volunteers (18-36 years) during selective attentional tasks following oral consumption of L-theanine (250 mg) or placebo. Theanine consumption resulted in an overall decrease in alpha activity while subjects were engaged in a very demanding cognitive task. However, alpha band activity was increased when subjects were under conditions requiring intersensory selective attention. The authors conclude that L-theanine may have specific effects on the brain's attention circuitry.

Since L-theanine is commonly consumed in conjunction with caffeine, clinical studies have been performed to assess the effects of L-theanine on cognition and attention in the presence and absence of caffeine. In a recent study, Owen *et al.* (2008) investigated the effect of caffeine (50 mg) on cognition and mood, in the presence and absence of L-theanine (100 mg). The authors found that the caffeine and theanine combination improved both speed and accuracy of performance on an attention-switching task at 60 minutes, and reduced susceptibility to distracting information in a memory task at both 60 and 90 minutes. The authors claimed that their results suggest L-theanine and caffeine together are beneficial for improving performance on cognitively demanding tasks.

In another randomized, double-blind, placebo-controlled, balanced crossover study, Haskell *et al.* (2008) investigated the acute cognitive and mood altering effects of L-theanine (250 mg) and caffeine (150 mg) alone and in combination. Experiments were performed with 24 volunteers, ages 18-34. The authors found that L-theanine alone had relatively few effects. Out of 38 cognitive measures performed, the authors found an effect of L-theanine on two. L-Theanine increased 'headache' ratings and decreased correct serial seven subtractions. Comparisons of the individual drinks to placebo showed that L-theanine alone significantly impaired performance on serial seven subtraction task at 90 min. The investigators suggested that beverages containing L-theanine and caffeine may have a different pharmacological profile to those containing caffeine alone.

In a third study assessing the effects of L-theanine in the presence and absence of caffeine, Kelly *et al.* (2008) administered 100 mg of L-theanine in the presence and absence of 50 mg of caffeine. In this study, 16 volunteers, ages 21-40 were given L-theanine and/or caffeine. Behavioral and electrophysiological analyses were performed. No effects on hit rate were observed, and no effect on tonic alpha amplitude was found with L-theanine alone. In contrast, a decrease in tonic alpha amplitude was found with the combination. In addition, the authors stated that previous unpublished studies of L-theanine at a higher dose of 250 mg also showed a decrease of tonic alpha amplitude. Based on their data, the authors suggested that L-theanine may work to enhance the tonic apportionment of attentional resources to the visual modality when administered at high levels or in combination with caffeine.

Numerous clinical studies measuring the anti-stress effects of L-theanine have been published. Kimura *et al.* (2007) performed a study of the effect of L-theanine on psychological and physiological stress responses in twelve male volunteers, ages 21-25. In this study, the authors

measured cardiodynamic activity utilizing ECG, and they measured volume of saliva and the concentration of s-IgA in saliva. Administration of L-theanine (200 mg, oral) to participants subjected to the stress of a mental arithmetic test showed non-significant increases in heart rate and reduced salivary IgA levels. The authors note that the small number of participants and the relatively few parameters measured limits the study. However, the investigators concluded that, with these given limitations, the administration of L-theanine reduced the stress response induced by the mental arithmetic test.

In a double-blind placebo-controlled repeated measures design, Lu *et al.* (2004) compared the acute effects of L-theanine with the benzodiazepine anxiolytic (anti-anxiety) alprazolam on behavioral measures of anxiety. In this study, sixteen volunteers were administered 200 mg of L-theanine, alprazolam (1 mg), or placebo. The effects of treatment were assessed under a relaxed and experimentally induced anxiety condition. The results showed some evidence for relaxing effects of L-theanine during the baseline condition on the tranquil-troubled subscale of the VAMS. Neither L-theanine nor alprazolam had any significant anxiolytic effects during the experimentally induced anxiety state. The findings from this study suggest that, while L-theanine may have some relaxing effects under resting conditions, L-theanine or alprazolam did not demonstrate any acute anxiolytic effects under conditions of increased anxiety in the anticipatory anxiety model.

In addition to the above-described studies, summaries of a series of unpublished studies, abstracts, or foreign language articles are described in GRN 209. These summaries and findings described in GRN 209 are presented below.

In a randomized double-blind crossover study, 23 healthy male golfers received 300 mg Suntheanine® + 20 mg L-carnitine + 100 mg Dongchunghacho or a placebo for 2 days (Kim et al., 2001). Those receiving the theanine-containing beverage showed significant increases in alpha waves of the frontal and occipital regions beginning 30 minutes after the administration. Of most interest to golfers, there were significant improvements in putting success rate and in driving accuracy.

In another study, "Kobayashi et al. (1998) divided 50 females age 18-22 into a high-anxiety group and a low-anxiety group based on scores on the Manifest Anxiety Scale. Four high- and four low-anxiety subjects were given either 50 mg or 200 mg theanine in water once a week. Following the administration of theanine, each subject had an electroencephalogram (EEG) for 60 minutes. Effects were noted about 30-40 minutes after the intake with an increased production of alpha waves (reported to be characteristic of relaxation), but not theta waves (reported to be indicators of drowsiness) were observed.

In a randomized placebo-controlled double-blind crossover study, Song et al. (2002) investigated the effects of L-theanine in 20 subjects age 30-55 years suffering from persistent fatigue. The subjects were classified with high or low anxiety and were administered a placebo or 200 mg L-theanine every day for 7 days. Each subject's frontal and occipital EEG was measured over 1 hour immediately after the administration of L-Theanine. Results showed significant relaxant effects of theanine. In a similar follow-up randomized placebo-controlled double-blind

crossover study with 20 younger males (age 18-30), Song et al. (2003) noted significant ($p < 0.05$) differences in those with high anxiety, but not in those with low anxiety. The investigators concluded that the results of both studies indicate that theanine promotes brain activity related to mental relaxation and concentration.

Also summarized in GRN 209 (2006), Weiss and his colleagues" conducted a number of studies of the effects of L-theanine on the recovery of individuals from the stress of heavy exercise. In an unpublished study (Weiss et al. undated), 14 healthy male athletes exercised on a bicycle ergometer and then consumed a drink with 0, 50, or 200 mg L-theanine in a randomized double-blind crossover design. Theanine had no effect on hormones important for regeneration, but lowered prolactin (which is regulated by dopamine and serotonin). There was no significant change in brain function, but lowered β waves, reported to be an indication of relaxation, were observed. In a similar randomized double-blind crossover study, Weiss et al. (2001a) gave 14 healthy athletes drinks with 0, 50, or 200 mg theanine after exhausting exercise on a bicycle ergometer. L-Theanine had no effect on peripheral sympathetic electrodermal activity during regeneration. In another phase of this same study but reported separately (Weiss et al. 2001b), plasma levels of catecholamines, cortisol, prolactin, and serotonin were measured 0, 30, 45, 60, and 120 minutes after the drink. No effects of theanine on hormonal levels were observed. Finally, in a third phase of the study (Weiss et al. 2001c), EEG mapping was used to assess the effect of ingestion of L-theanine; the experimenters reported that L-theanine supported physiological relaxation after severe exercise.

In another unpublished study summarized in GRN 209, "Weiss and Geiss (undated) sought to determine if theanine administration had any adverse effects on cognitive function, such as poorer reaction time, concentration, alertness, or attention. In a randomized double-blind placebo-controlled crossover study, 20 male students age 19-32 ingested either placebo or 200 mg L-theanine at breakfast. No significant differences were observed in heart rate or blood pressure, blood glucose, red blood cells, hemoglobin, hematocrit, uric acid, urea, or γ -glutathione. No differences were observed in reaction time or accuracy of response, concentration, or perceptual speed on a tachistoscopic test.

VI. DISCUSSION OF REVIEWED INFORMATION & GRAS CRITERIA

A. GRAS Criteria

FDA defines “safe” or “safety” as it applies to food ingredients as

“...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.”⁴

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA’s operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

“...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.”

“General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information.”⁵

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, the so-called “common knowledge element,” in terms of the two following elements:⁶

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

⁴ See 21CFR 170.3(i).

⁵ See 21CFR 170.30(a).

⁶ See footnote 1.

The apparent imprecision of the terms “appreciable”, “at the time” and “reasonable certainly” demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu, 1988; Renwick, 1990).

B. Discussion of *L-Theanine* ($\geq 98\%$) Safety

A significant amount of information related to the safety of L-theanine is available for evaluation. In addition to a long history of safe consumption of L-theanine from its natural presence in tea, the safety of L-theanine has been the subject of a number of experimental studies in both animals and humans. The evidence from historical safe consumption of L-theanine from tea and experimental studies together can be used to determine its safe use for human consumption.

As noted in Section V, tea is the only naturally occurring source of theanine in the diets of Americans and, in fact, of most people around the world. Around the world, tea is the most widely consumed beverage after water. In the US, as a beverage tea competes with coffee and a broad selection of other beverages. Available surveys suggest that approximately 20% of adults in the United States are reported to consume tea regularly. The mean amount of tea beverage consumed in the US is ~800mL/person, while the 90th percentile consumers ingested 1650 mL of tea, and the 99th percentile consumers' intake has been estimated as 3300 mL/person. It is possible that in countries, particularly in Asia, where tea consumption is very common, the per capita tea consumption is likely to be even higher. The theanine content of a broad selection of tea leaves has been reported to range from 1 to 2.5%. Given high water solubility of theanine, nearly all of the theanine from tea leaves during tea preparation is likely to dissolve in the water and be consumed. The estimated intake of theanine by tea drinkers has been estimated to be 152-382 mg/day at the mean, between 330 and 825 mg/day at the 90th percentile, and as much as 667-1668 mg/day at the 99th percentile. Thus, the available exposure information suggests safe consumption of tea, which, in turn, supports the safety of theanine consumption from its presence in tea.

L-Theanine has been well studied in laboratory animal toxicity investigations and in clinical studies with human subjects. Available evidence from metabolism and kinetic studies suggest that, following oral ingestion, theanine is absorbed in the blood from the intestines and is distributed in many tissues, such as the liver and brain. Inside the human body, theanine is hydrolyzed to glutamine and ethylamine. Within a few hours of ingestion, both theanine and its metabolites reach peak concentrations in the plasma and in tissues followed by rapid decline, along with a concomitant increase in urinary concentrations. No evidence exists to suggest that L-theanine would bioaccumulate.

In acute toxicity studies, the oral LD₅₀ of L-theanine has been reported to be >5000mg/kg bw suggesting that L-theanine is practically non-toxic (Derelanko and Hollinger, 1995). In a short-term (28-day) oral toxicity study, the NOAEL at the only dose tested, was 2000 mg/kg bw/day. In a 13-week subchronic dietary toxicity study, rats were fed diet containing 1500, 3000, or 4000 mg theanine/kg bw/day and no toxicity was noted at any tested dose. The results of this study suggest that the NOAEL was the highest dose of 4000 mg/kg bw/day. In this study, renal tubular cell

adenomas were found in two high-dose and one mid-dose treated female rats. Further extensive investigation revealed that these tumors were not treatment-related but were due to an unusual genetic anomaly among the population of rats used in this study. No such changes were noted in subchronic and chronic studies in mice. Additionally the long history of safe consumption of tea and epidemiological evidence does not support any association between L-theanine and renal tubular cell adenomas. The Panel agrees that the kidney tumors noted in the rat study were an anomalous occurrence and have no bearing on the safety assessment of theanine. In a subchronic (13 weeks) and chronic (78 weeks) study of L-theanine in mice, no evidence of toxicity was noted. The results of the chronic study in mice showed no evidence of carcinogenicity of L-theanine. In genetic toxicology tests, L-theanine showed no mutagenic or clastogenic activity, further indicating that L-theanine lacks the potential to be carcinogenic. In clinical trials with human volunteers studying various possible benefits of L-theanine, no adverse effects were noted.

The no adverse effect level of 4000 mg/kg bw/day from the subchronic study in rats corresponds to a dose level of 240 g/day for an individual weighing 60 kg. The safety studies as corroborated by history of use information support the safety-in-use determination at the intended use levels. The Panel has concluded that the proposed average consumption of 0.63 g/person/day or 11 mg/kg bw/day and maximum consumption of approximately 1.28 g/person/day or 24 mg/kg bw/day from the use in the food categories proposed in Section IV is safe. The Panel has also determined that there is an approximate margin of safety of over 100-fold from the doses found to have no effect in long term studies in mice and rats.

Sufficient qualitative and quantitative scientific evidence exists, including human and animal data, to support the safety-in-use of *L-theanine* ($\geq 98\%$). The evidence of *L-theanine* ($\geq 98\%$) safety is underscored by virtue of the following:

- L-Theanine, an amino acid, is found in free form in tea and has a long safe history of human consumption.
- L-Theanine is isolated from tea leaves (*Camellia sinensis*) in accordance with current Good Manufacturing Practices.
- There is no evidence that consumption of L-theanine either in foods or as a dietary supplement has adverse effects.
- The bioavailability of the ingested L-theanine is limited as it is metabolized and rapidly excreted with no detectable levels in blood plasma 6 hours after oral administration.
- The metabolism of L-theanine has been well characterized, and neither L-theanine nor its metabolites bioaccumulate.
- A variety of animal, human and *in vitro* studies support the safety of L-theanine.

In addition, the manufacturing procedures and specifications established by Tianrui Chemical are adequate to define a suitable purity to be considered food-grade.

C. Common Knowledge Elements

The first common knowledge element for a GRAS determination requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The majority of the studies reviewed in this safety assessment have been published in the scientific literature as reported in Section V. Specifically, studies by Borzelleca *et al.* (2006), Hall *et al.* (2007) and Fujii and Inai (2008) have investigated the subchronic and chronic toxicity of L-theanine in rats and mice. Findings from these investigations have been published in peer reviewed journals that are readily available. In addition to the many scientific studies that have been conducted and published, history of tea consumption and, in turn, intake of L-theanine since ancient times is well known around the world.

Furthermore, safety documentation for food uses of L-theanine is found in GRN 209, which also constitutes information that is generally available for review and evaluation. The composite information noted thereby fulfills the common knowledge element required for GRAS determinations.

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VII. CONCLUSIONS

Based on the information provided herein, Zhejiang Tianrui Chemical Co., Ltd. (Zhejiang Province, China), also referred to as Tianrui Chemical, has determined through scientific procedures that the use of its manufactured ingredient, *L-theanine* ($\geq 98\%$), in food for humans is generally recognized as safe (GRAS) in accordance with Section 201(s) of the federal food, Drug, and Cosmetic Act.

L-Theanine ($\geq 98\%$) is intended for use as an ingredient in fruit juices and drinks, non-herbal teas, sports beverages, bottled waters, chocolate bars and chews, hard candies, breath mints, and chewing gum at levels up to 250 mg per serving. Identical food uses and levels were previously described in two prior documents filed as GRN No. 209 submitted by Taiyo International, Inc. and GRN No. 338 submitted by Blue California. In both cases, FDA indicated based on the available information that it had no questions regarding each company's conclusion that *L-theanine* is GRAS under the intended conditions of use.

Since Tianrui Chemical intends to use *L-Theanine* ($\geq 98\%$) in the same foods and at the same levels as the two prior notices, the present notice relies on these documents as the basis for the conclusion that the use of *L-Theanine* ($\geq 98\%$) is likewise GRAS. Tianrui Chemical's *L-Theanine* ($\geq 98\%$) is different in that it is chemically-synthesized from L-glutamic acid and ethylamine, rather than being derived enzymatically (GRN No. 209) or extracted from tea leaves (GRN No. 338).

Tianrui Chemical also sought the opinion of David Bechtel, PhD, DABT, an expert in food toxicology and regulatory affairs, affiliated with Intertek Cantox, located at 1011 US Highway 22, Suite 200, Bridgewater, NJ 08807. His expert opinion statement is attached as Appendix C.

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APPENDIX A

CHEMICAL IDENTIFICATION OF MULTIPLE LOTS OF TIANRUI CHEMICAL'S L-THEANINE INGREDIENT

Lot Numbers: TR20120815

TR20131015

TR20121119

TR20130625

TR20130925

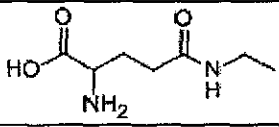
000051

SHANG PHARMA CORPORATION SHANGHAI CHEMPARTNER CO., LTD

RECORD OF ANALYSIS

No.:COA-TR-001

☒ Drug Active ☐ Intermediate ☐ Impurity ☐ Other:
Intended Use: ☒ Quantitative ☒ Qualitative ☐ Limit Test

Chemical Name: DL-THEANINE; H-DL-The-OH; 2-Amino-4-(ethylcarbamoyl)butanoic acid Other Name: L-Theanine	
Description: N/A	Manufacturer: N/A. Manufacturer Lot Number: TR20120815 TR20131015 TR20121119 TR20130625 TR20130925 Quantity: N/A
Effective Date: Oct-30-2013 Analysis Date: Oct-22-2013 Date Received: Oct-21-2013	

Tests	Results				
	TR20120815	TR20131015	TR20121119	TR20130625	TR20130925
1. Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder
2. Identification by IR	The spectra of the sample conforms to that of a reference standard	The spectra of the sample conforms to that of a reference standard	The spectra of the sample conforms to that of a reference standard	The spectra of the sample conforms to that of a reference standard	The spectra of the sample conforms to that of a reference standard
3. Assay%	99.44	99.80	100.79	100.75	101.39
4. Optical Rotation*	8.458	8.249	7.920	8.199	8.295

*Reference Standard(Lot#:LE10103): Optical Rotation = 8.259

Prepared by (b) (6) Date Oct-30-2013
Xiaowei Gu, Scientist, Pharmaceutical Development Service Center

Reviewed by (b) (6) Date Oct-30-2013
Bangjie Dong, Team Leader, Pharmaceutical Development Service Center

Approved by (b) (6) Date Oct-30-2013
Qichun Mi, Manager, Quality Assurance

SHANG PHARMA CORPORATION SHANGHAI CHEMPARTNER CO., LTD

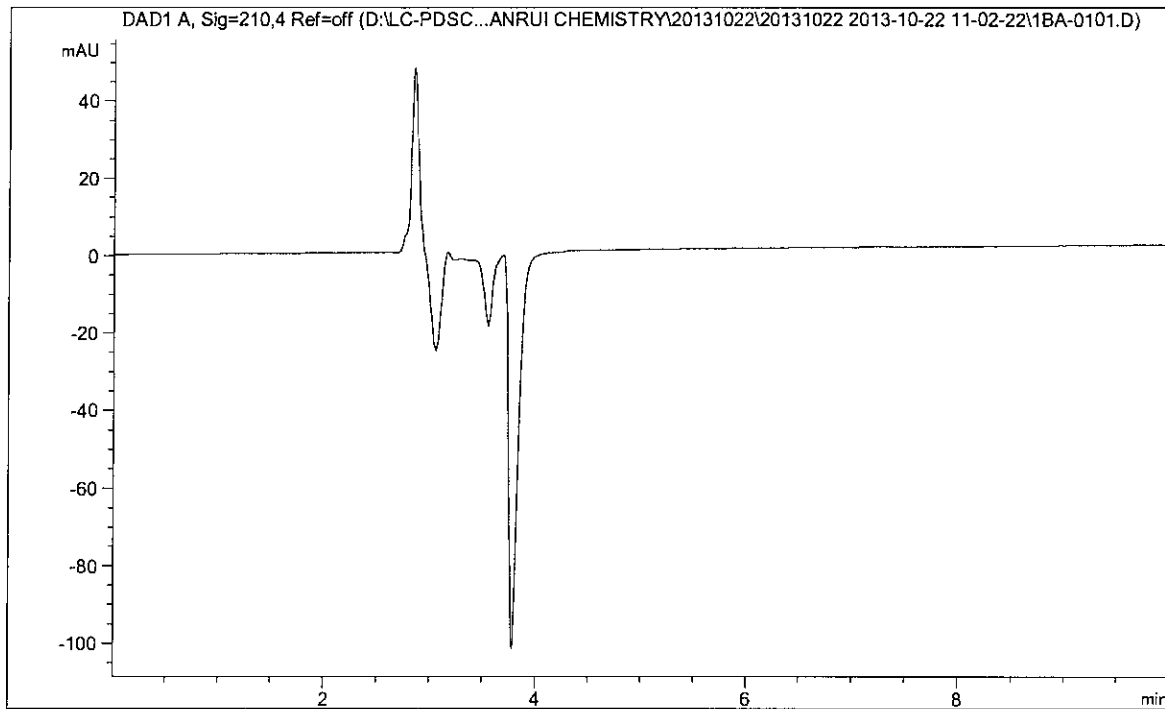
REFERENCE:

Tests Performed	Reference (NB assignee)
Appearance	NB-CP-003252-098~101
Identification by IR	NBK-QC010-V01-015-19~21 NBK-QC010-V01-015-27~28
Assay	NB-CP-003252-098~101
Optical Rotation	PDSC-NB-SR-01-1-031~036

DOCUMENT HISTORY:

Version	Document Number	Comments
1	COA-TR-001	New Document

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                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M.
Last changed    : 2013-10-22 4:13:23 PM by zt
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=====
                          Area Percent Report
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Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
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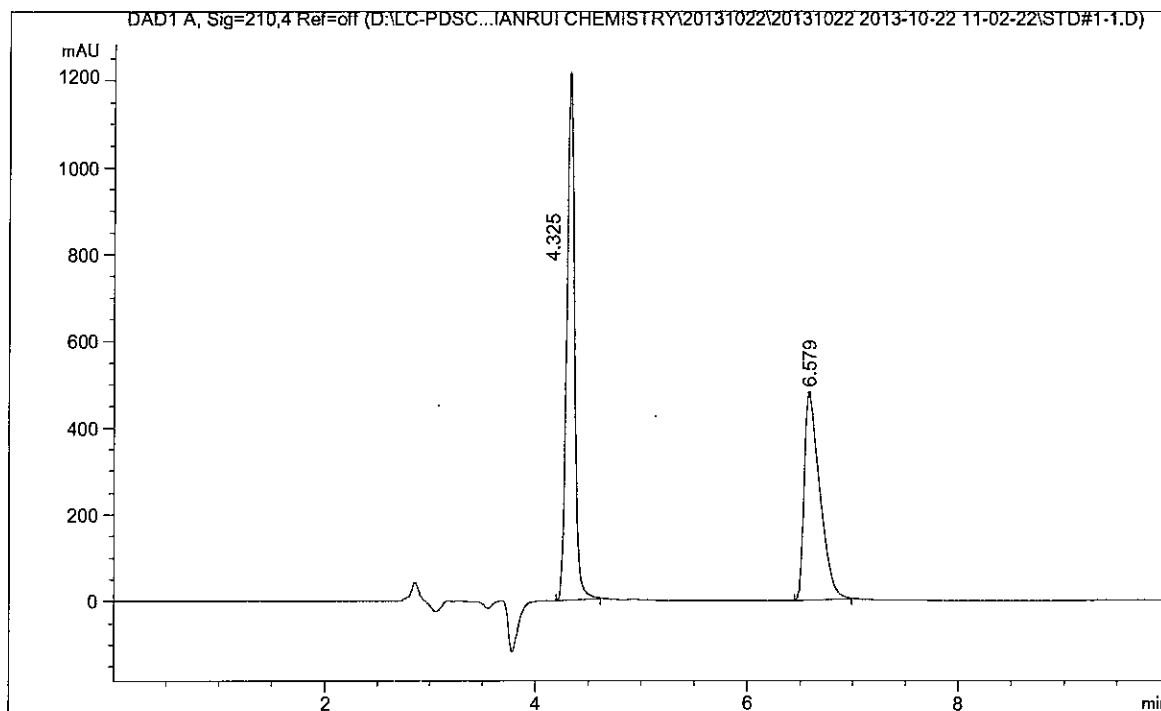
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000054

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                                           Inj Volume: 10 µl
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                22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
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Last changed    : 2013-10-22 4:15:38 PM by zt
                (modified after loading)
=====
```

Maximum Pressure : 400 bar Maximum pH : 9
Maximum Temperature: 60 °C
Comment :



```
=====
Area Percent Report with Performance and Noise
=====
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Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

000055

Signal 1: DAD1 A, Sig=210,4 Ref=off

Noise determination:

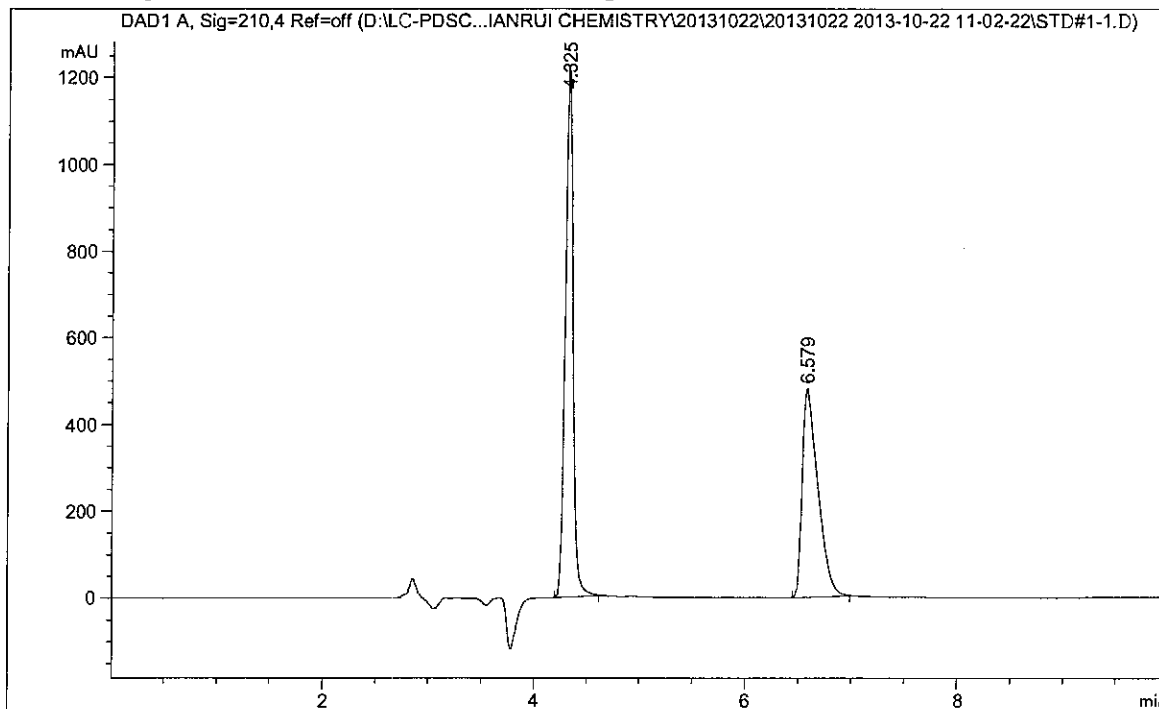
Time range		Noise	Noise	Noise		
from	to	(6*SD)	(PtoP)	(ASTM)	Wander	Drift
[min]	[min]	[mAU]	[mAU]	[mAU]	[mAU]	[mAU/h]
8.000	9.000	0.1129	8.086e-2	-	-	3.866

RetTime	k'	Area	Height	Symm.	Width	Plates	Resol	Signal
[min]		[mAU*s]	[mAU]		[min]		ution	/Noise
4.325	-	6292.76123	1220.12927	0.86	0.0767	17629	-	1.1e4
6.579	0.32	4947.57715	479.89093	0.39	0.1567	9769	11.35	4252.0

*** End of Report ***

000056

```
=====
Acq. Operator   : gxw                      Seq. Line :    2
Acq. Instrument : LC-PDSC-06              Location  : Pl-B-02
Injection Date  : 2013-10-22 11:13:34 AM Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



```
=====
                          Area Percent Report
=====
```

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

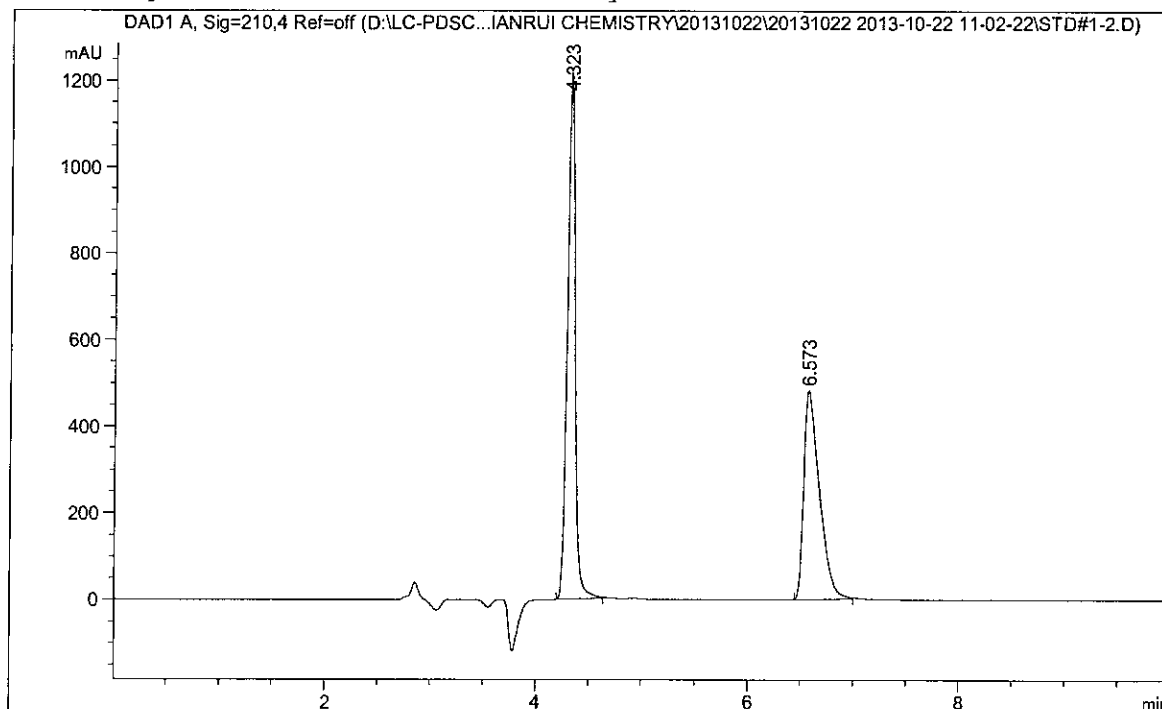
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.325	BB	0.0802	6292.76123	1220.12927	55.9837
2	6.579	BB	0.1523	4947.57715	479.89093	44.0163

Totals : 1.12403e4 1700.02020

000057

```
=====
Acq. Operator   : gxw                      Seq. Line :    2
Acq. Instrument : LC-PDSC-06              Location  : P1-B-02
Injection Date  : 2013-10-22 11:24:32 AM   Inj       :    2
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



Area Percent Report

```
=====
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====
```

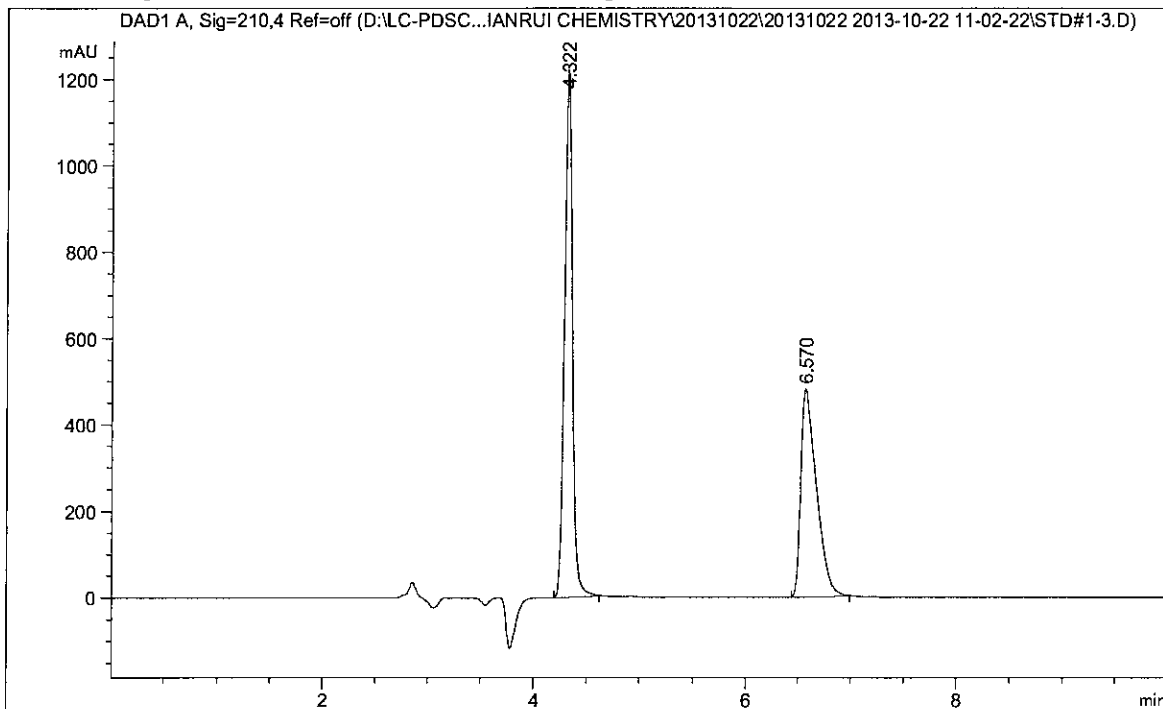
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.323	BB	0.0803	6307.45459	1219.59912	56.0270
2	6.573	BB	0.1501	4950.42383	480.83057	43.9730

Totals : 1.12579e4 1700.42969

000058

```
=====
Acq. Operator   : gxw                      Seq. Line :    2
Acq. Instrument : LC-PDSC-06              Location  : P1-B-02
Injection Date  : 2013-10-22 11:35:31 AM   Inj       :    3
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



```
=====
                          Area Percent Report
=====
```

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 A, Sig=210,4 Ref=off

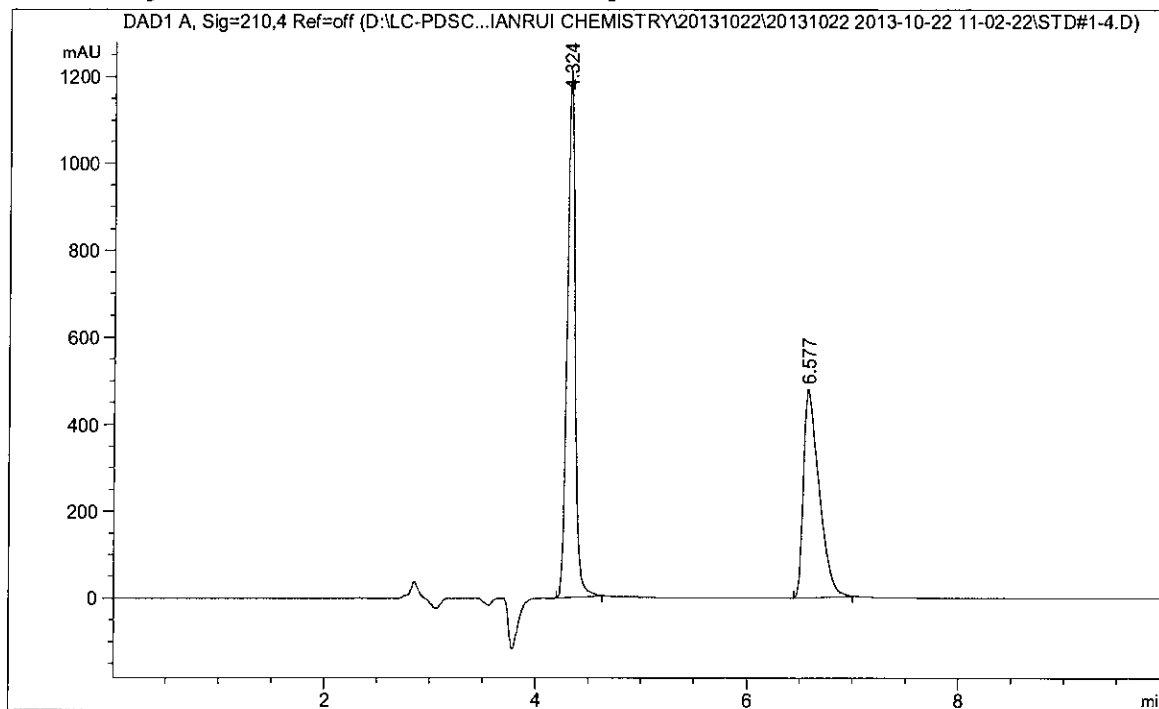
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.322	BB	0.0783	6306.66016	1220.41455	56.0101
2	6.570	BB	0.1522	4953.19678	480.94113	43.9899

Totals : 1.12599e4 1701.35568

000059

=====

Acq. Operator	: gxw	Seq. Line	: 2
Acq. Instrument	: LC-PDSC-06	Location	: P1-B-02
Injection Date	: 2013-10-22 11:46:30 AM	Inj	: 4
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

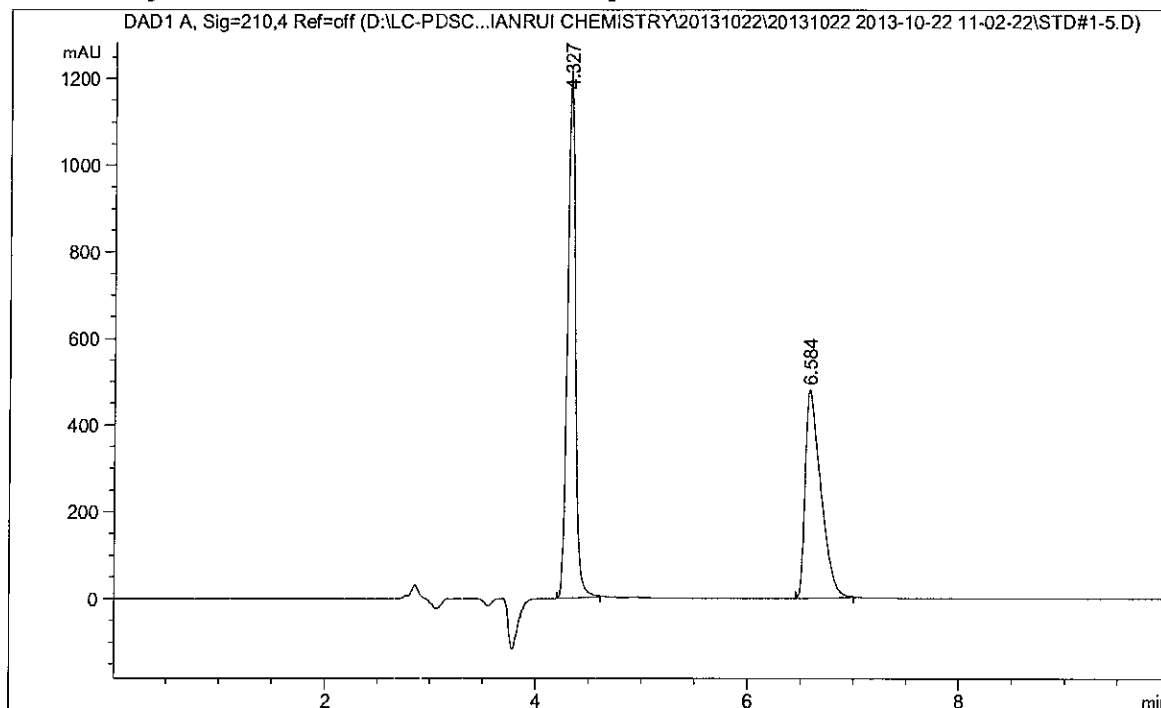
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.324	BB	0.0804	6296.19971	1216.82251	56.0286
2	6.577	BB	0.1525	4941.27734	478.40939	43.9714

Totals : 1.12375e4 1695.23190

000060

```
=====
Acq. Operator   : gxw                      Seq. Line :    2
Acq. Instrument : LC-PDSC-06              Location  : P1-B-02
Injection Date  : 2013-10-22 11:57:26 AM   Inj       :    5
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



=====
Area Percent Report
=====

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 A, Sig=210,4 Ref=off

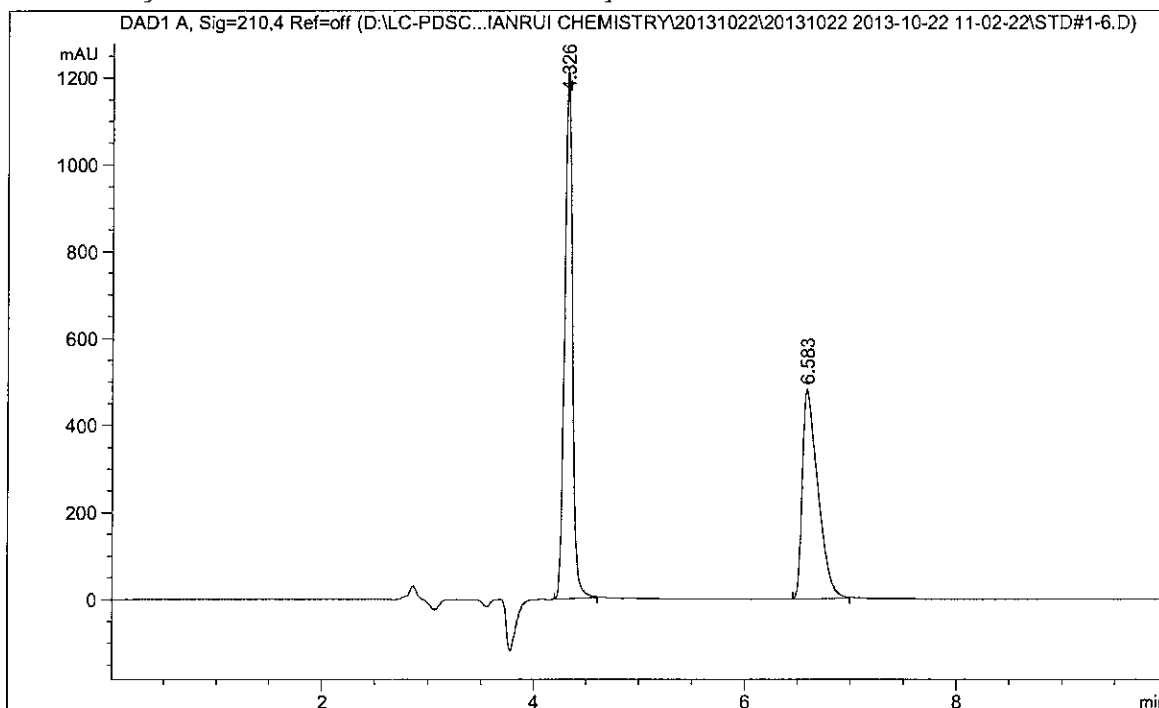
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.327	BB	0.0803	6292.44092	1218.24316	55.9274
2	6.584	BB	0.1526	4958.64600	479.70694	44.0726

Totals : 1.12511e4 1697.95010

000061

=====

Acq. Operator	: gxw	Seq. Line	: 2
Acq. Instrument	: LC-PDSC-06	Location	: P1-B-02
Injection Date	: 2013-10-22 12:08:26 PM	Inj	: 6
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

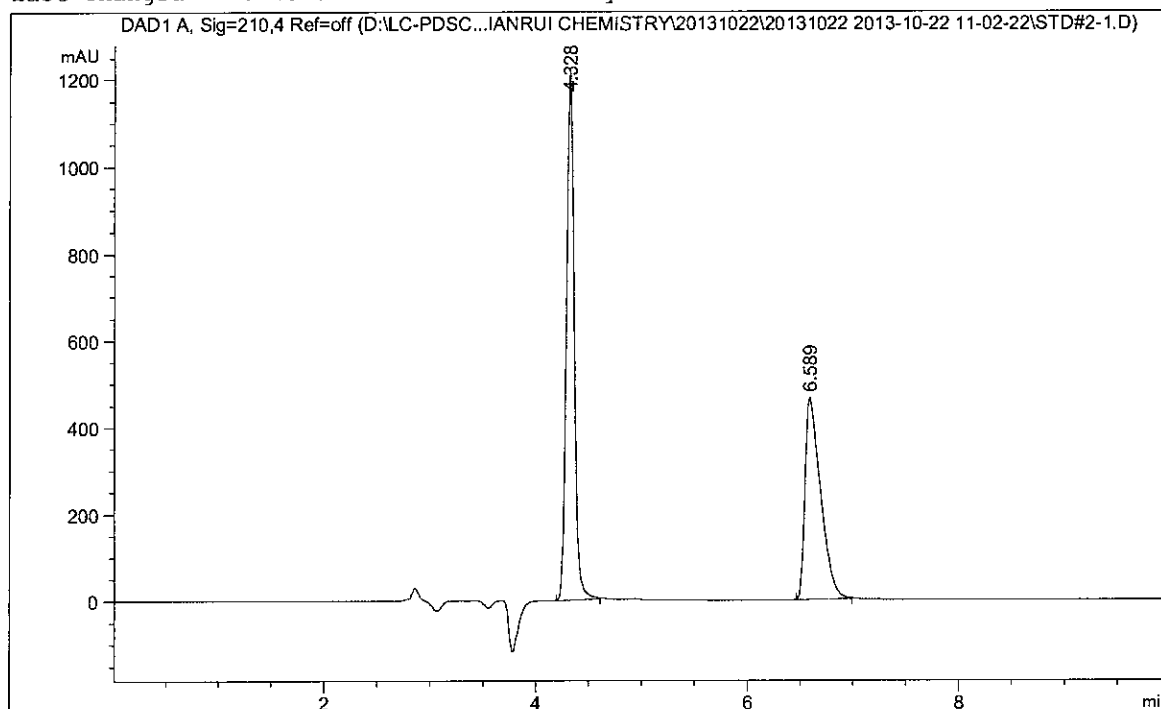
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.326	BB	0.0802	6278.32715	1217.53101	55.9634
2	6.583	BB	0.1503	4940.29590	479.30420	44.0366

Totals : 1.12186e4 1696.83521

000062

```
=====
Acq. Operator   : gxw                      Seq. Line :    3
Acq. Instrument : LC-PDSC-06              Location  : Pl-B-03
Injection Date  : 2013-10-22 12:19:25 PM   Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



```
=====
                          Area Percent Report
=====
```

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

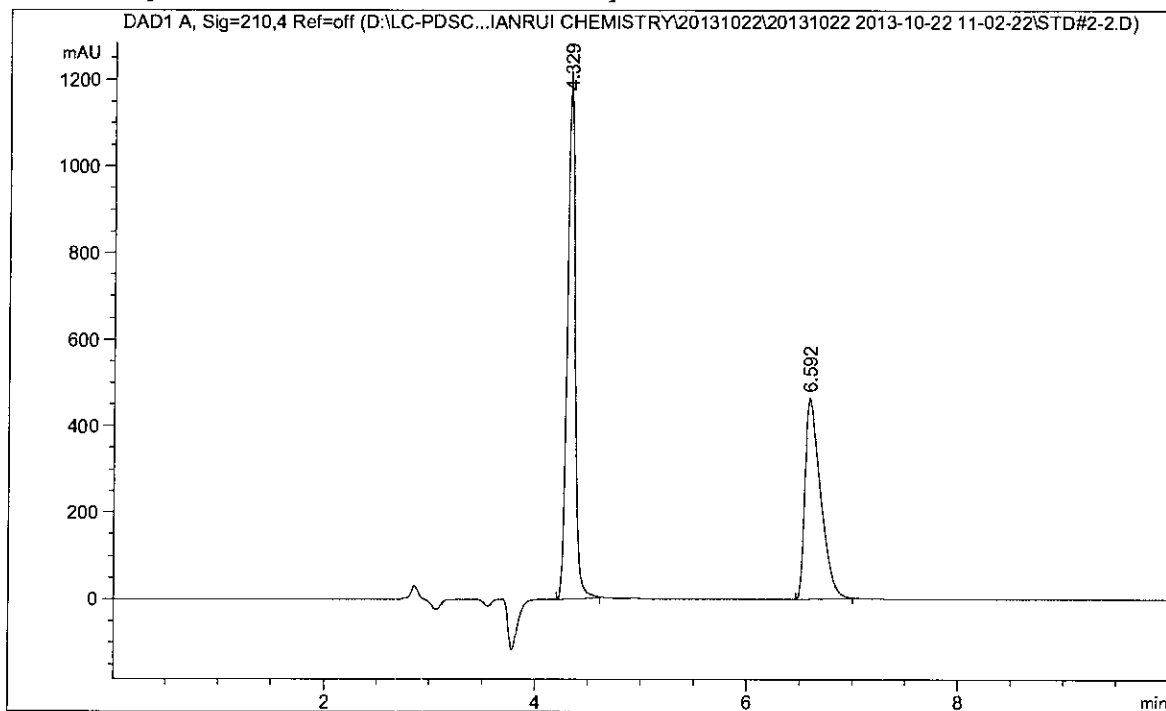
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.328	BB	0.0802	6274.91943	1216.40063	56.9218
2	6.589	BB	0.1490	4748.83594	465.41986	43.0782

Totals : 1.10238e4 1681.82050

000063

```
=====
Acq. Operator   : gxw                      Seq. Line :    3
Acq. Instrument : LC-PDSC-06              Location  : P1-B-03
Injection Date  : 2013-10-22 12:30:25 PM Inj       :    2
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



Area Percent Report

```
=====
Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
=====
```

Signal 1: DAD1 A, Sig=210,4 Ref=off

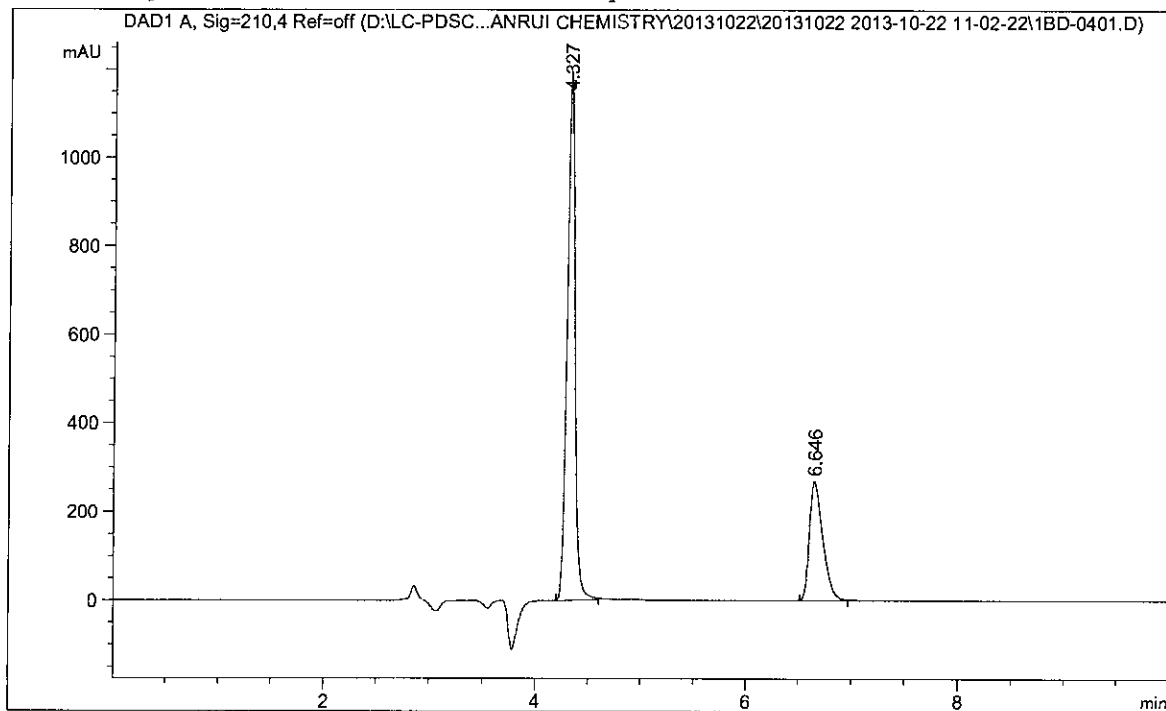
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.329	BB	0.0783	6290.91846	1217.53748	56.9228
2	6.592	BB	0.1514	4760.74658	465.07132	43.0772

Totals : 1.10517e4 1682.60880

000064

=====

Acq. Operator	: gxw	Seq. Line	: 4
Acq. Instrument	: LC-PDSC-06	Location	: P1-B-04
Injection Date	: 2013-10-22 12:41:24 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.327	BB	0.0803	6204.34814	1200.32117	72.2012
2	6.646	BB	0.1332	2388.78882	269.98224	27.7988

Totals : 8593.13696 1470.30341

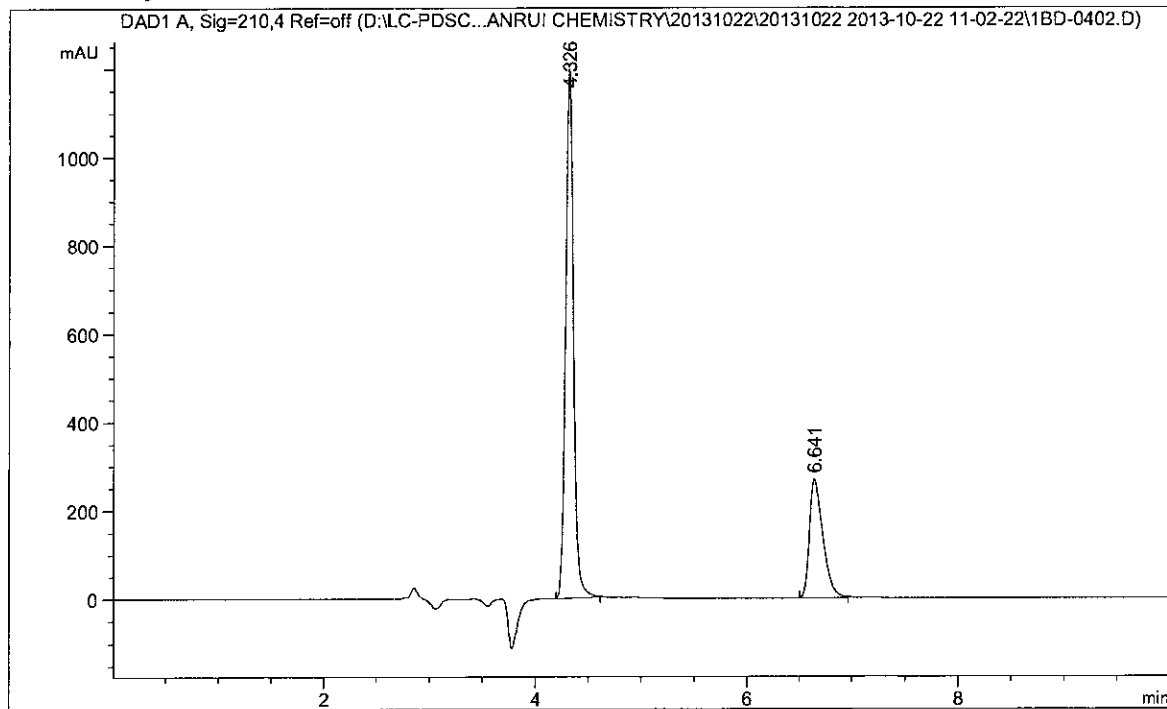
000065

Sample Name: L1

```

=====
Acq. Operator   : gxw                      Seq. Line :    4
Acq. Instrument : LC-PDSC-06              Location  : P1-B-04
Injection Date  : 2013-10-22 12:52:24 PM   Inj       :    2
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-
                                           22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====

```



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=====
                          Area Percent Report
=====

```

```

Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs

```

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width (min)	Area [mAU*s]	Height [mAU]	Area %
1	4.326	BB	0.0805	6221.15869	1198.69714	72.2469
2	6.641	BB	0.1335	2389.81079	269.36194	27.7531

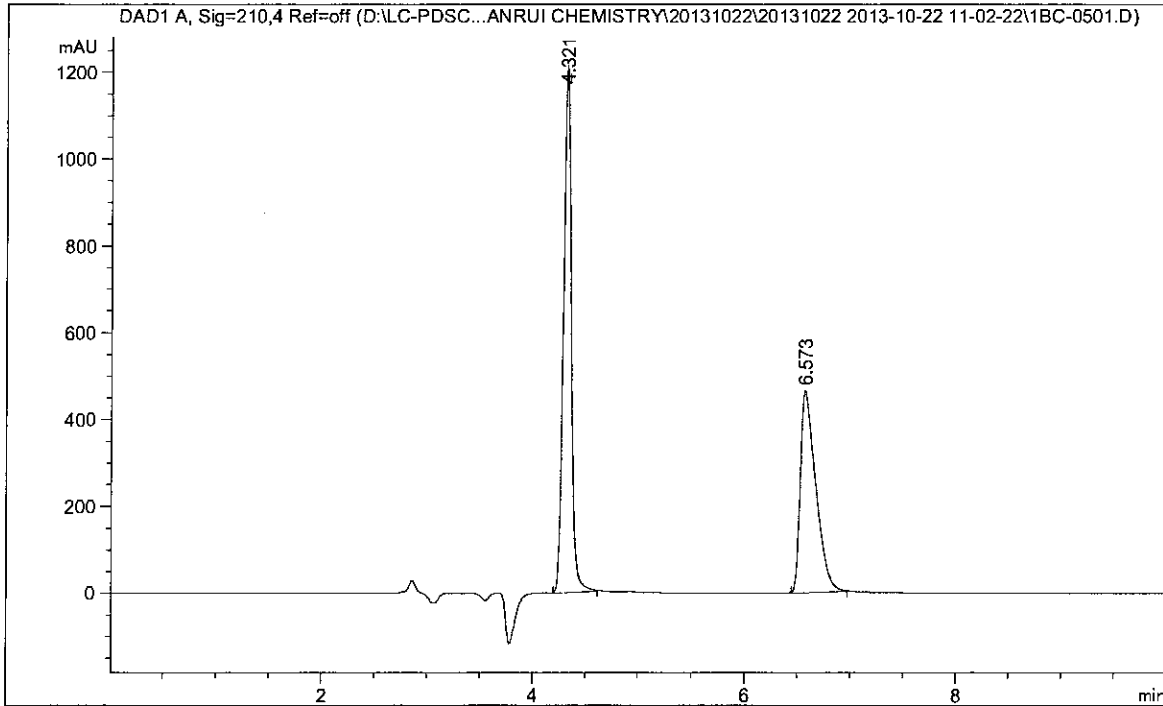
Totals : 8610.96948 1468.05908

000066

Page 63

=====

Acq. Operator	: gxw	Seq. Line	: 5
Acq. Instrument	: LC-PDSC-06	Location	: Pl-B-03
Injection Date	: 2013-10-22 1:03:24 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

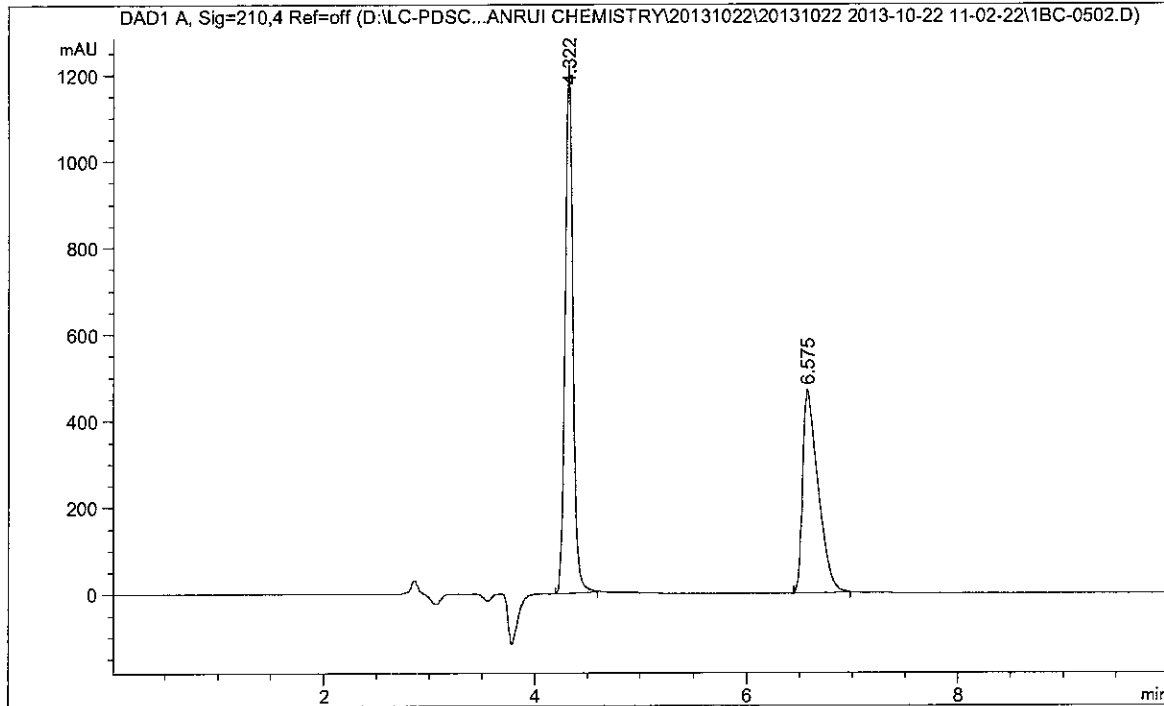
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.321	BB	0.0782	6269.93896	1214.65247	57.0159
2	6.573	BB	0.1486	4726.88525	464.94553	42.9841

Totals : 1.09968e4 1679.59799

000067

=====

Acq. Operator	: gxw	Seq. Line	: 5
Acq. Instrument	: LC-PDSC-06	Location	: P1-B-03
Injection Date	: 2013-10-22 1:14:23 PM	Inj	: 2
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

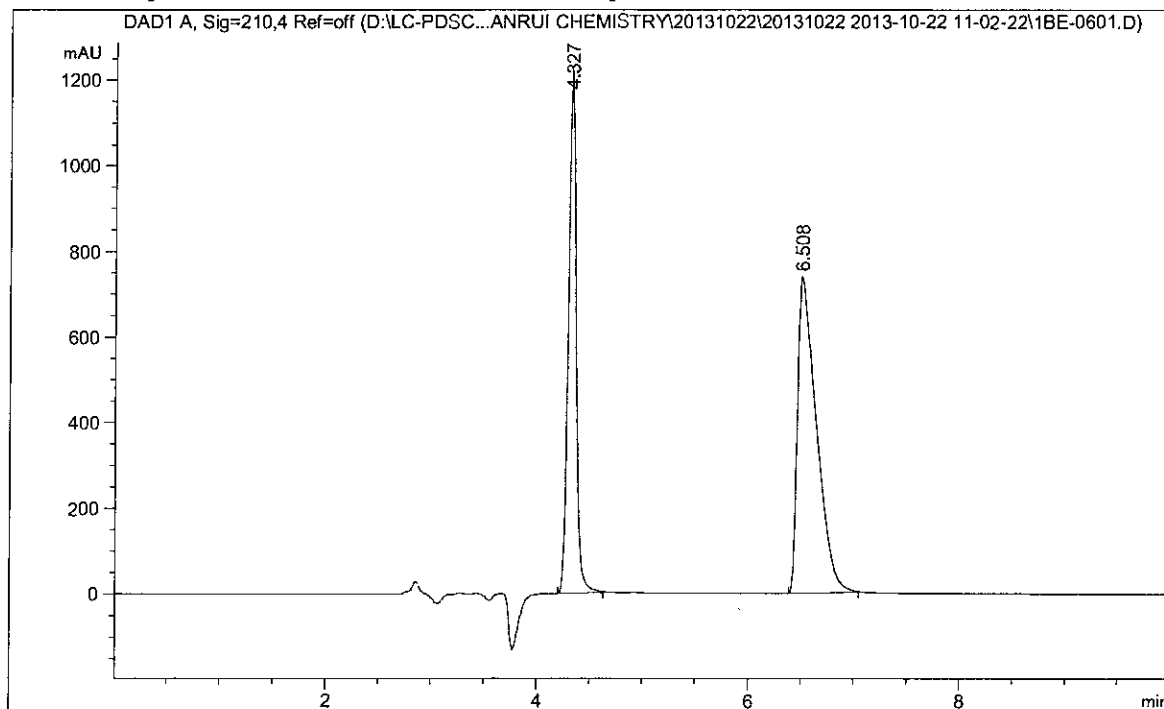
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.322	BB	0.0780	6268.98877	1218.14062	56.8755
2	6.575	BB	0.1508	4753.31982	466.89703	43.1245

Totals : 1.10223e4 1685.03766

000068

```
=====
Acq. Operator   : gxw                      Seq. Line :    6
Acq. Instrument : LC-PDSC-06              Location  : P1-B-05
Injection Date  : 2013-10-22 1:25:21 PM    Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

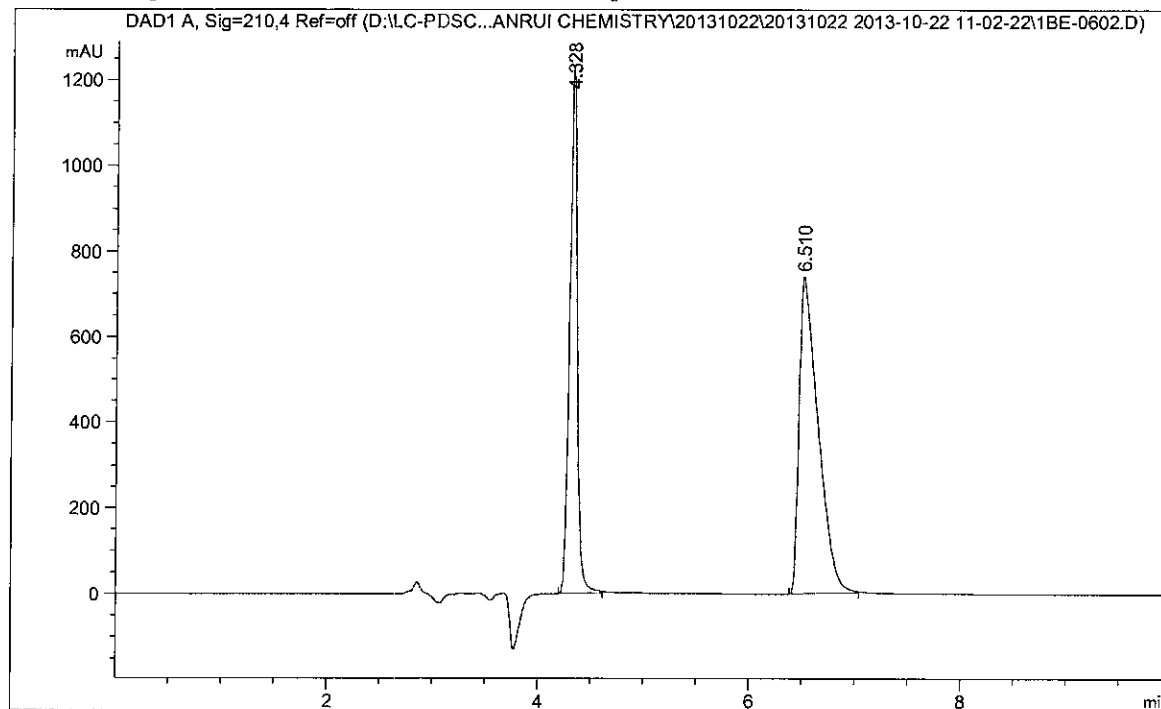
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.327	BB	0.0780	6275.91895	1219.63721	40.5367
2	6.508	BB	0.1811	9206.15137	740.34845	59.4633

Totals : 1.54821e4 1959.98566

000069

```
=====
Acq. Operator   : gxw                      Seq. Line :    6
Acq. Instrument : LC-PDSC-06              Location  : P1-B-05
Injection Date  : 2013-10-22 1:36:18 PM    Inj       :    2
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-
                                           22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

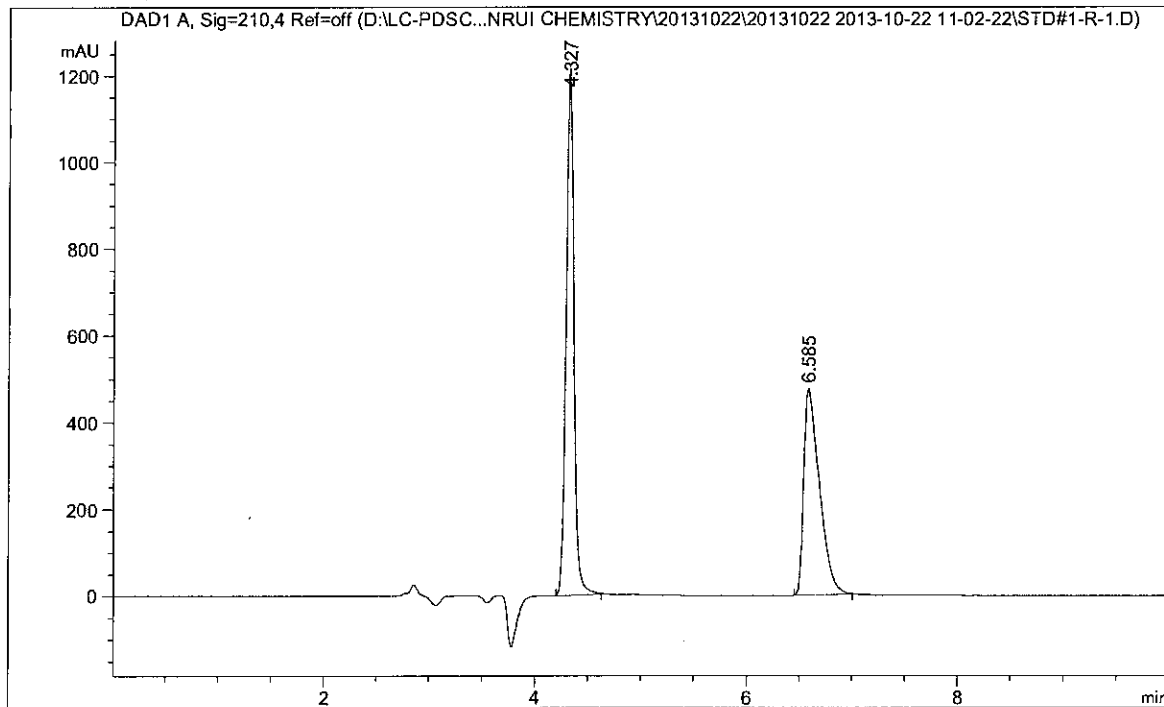
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.328	BB	0.0779	6266.14795	1220.95825	40.5009
2	6.510	BB	0.1811	9205.48340	740.23236	59.4991

Totals : 1.54716e4 1961.19061

000070

=====

Acq. Operator	: gxw	Seq. Line	: 7
Acq. Instrument	: LC-PDSC-06	Location	: P1-B-02
Injection Date	: 2013-10-22 1:47:18 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

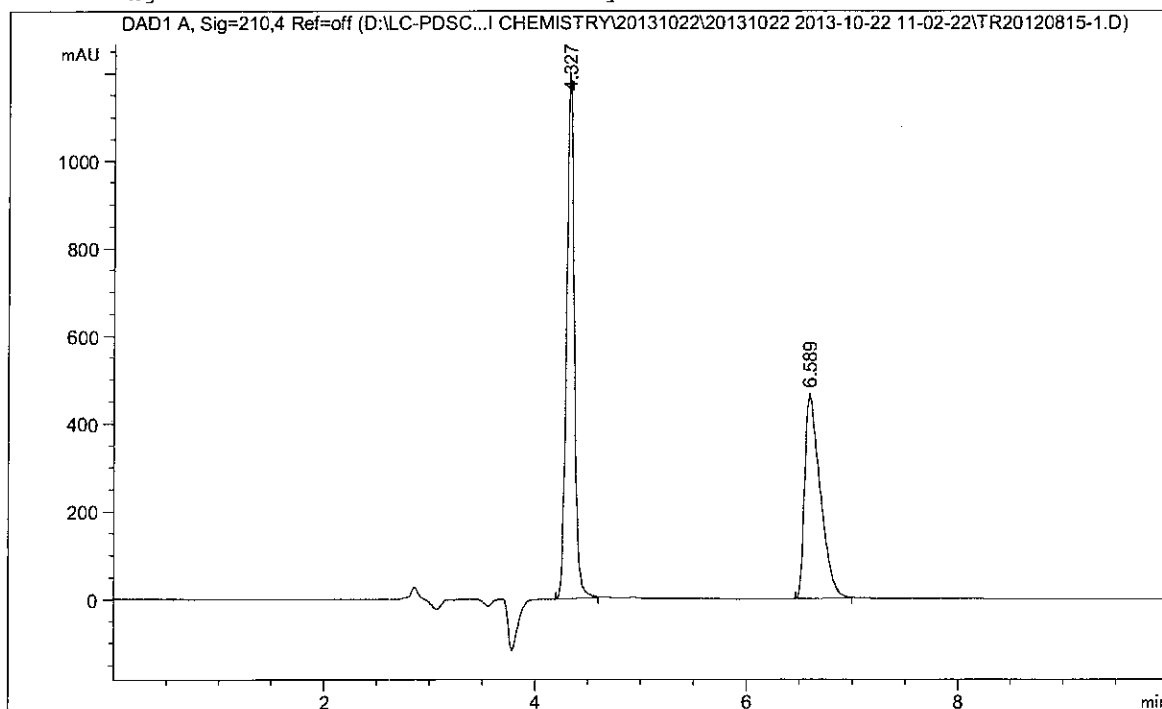
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.327	BB	0.0805	6308.08057	1215.88831	56.0308
2	6.585	BB	0.1530	4950.16602	477.27393	43.9692

Totals : 1.12582e4 1693.16223

000071

```
=====
Acq. Operator   : gxw                      Seq. Line :    8
Acq. Instrument : LC-PDSC-06              Location  : P1-B-06
Injection Date  : 2013-10-22 1:58:18 PM    Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-
                                           22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



```
=====
                          Area Percent Report
=====
```

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

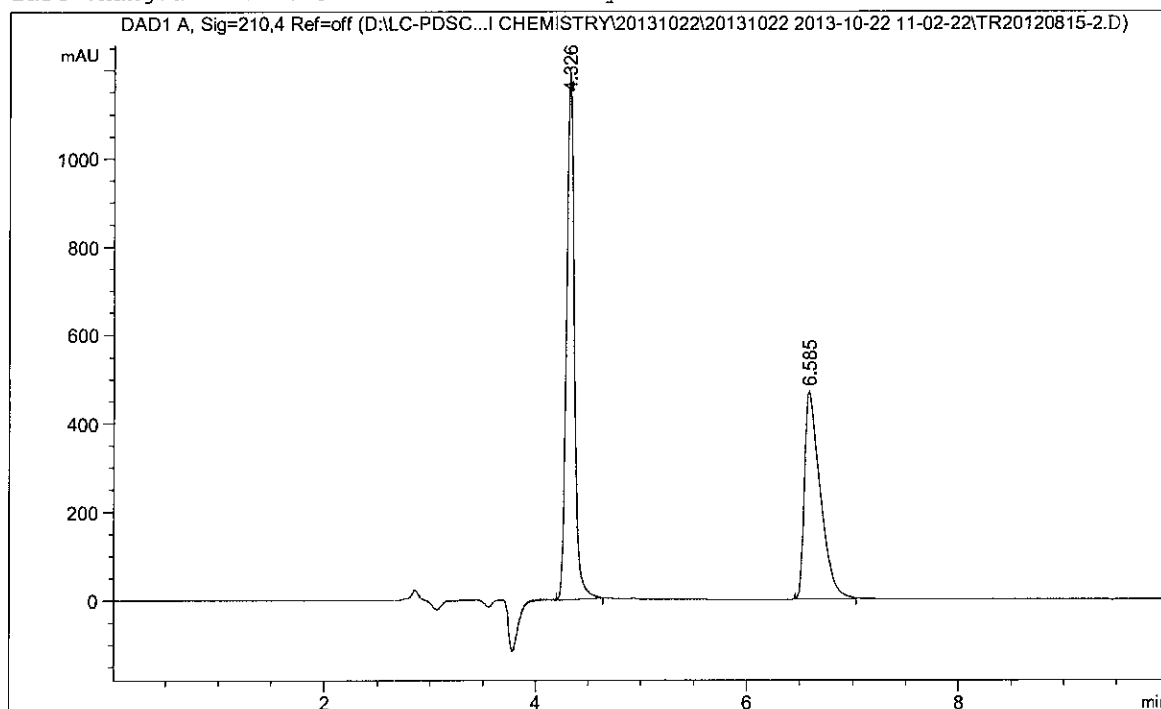
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.327	BB	0.0781	6188.74707	1200.69482	56.4637
2	6.589	BB	0.1512	4771.82471	467.05643	43.5363

Totals : 1.09606e4 1667.75125

000072

```
=====
Acq. Operator   : gxw                      Seq. Line :    9
Acq. Instrument : LC-PDSC-06              Location  : P1-B-07
Injection Date  : 2013-10-22 2:09:15 PM    Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



Area Percent Report

```
=====
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====
```

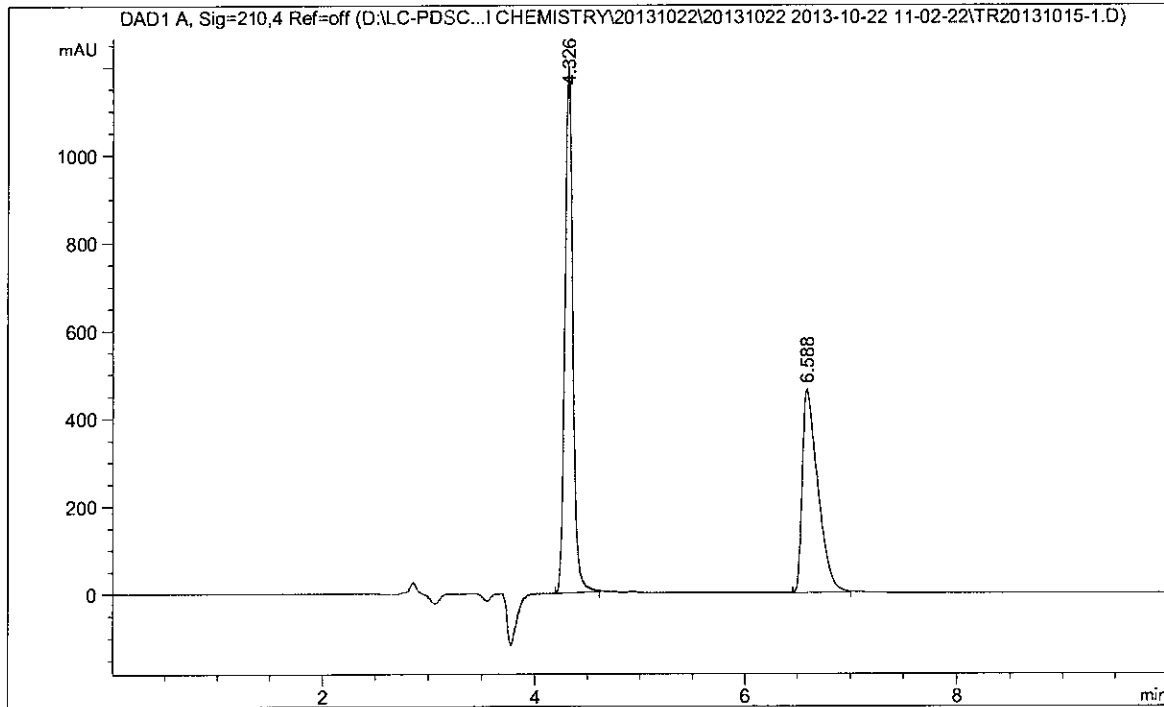
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.326	BB	0.0809	6242.51172	1196.51331	56.1731
2	6.585	BB	0.1531	4870.47070	469.32162	43.8269

Totals : 1.11130e4 1665.83493

000073

```
=====
Acq. Operator   : gxw                      Seq. Line :   10
Acq. Instrument : LC-PDSC-06              Location  : P1-B-08
Injection Date  : 2013-10-22 2:20:15 PM    Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-
                                           22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



=====
Area Percent Report
=====

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 A, Sig=210,4 Ref=off

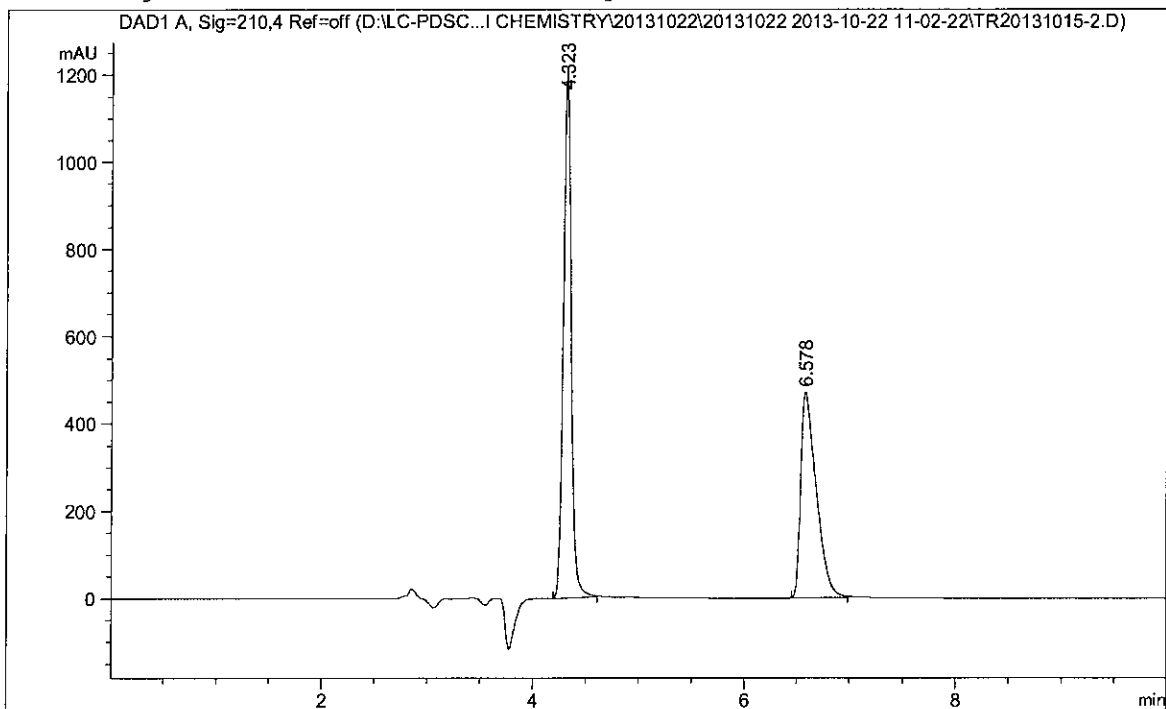
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.326	BB	0.0805	6231.20557	1202.36694	56.6663
2	6.588	BB	0.1517	4765.10498	464.30109	43.3337

Totals : 1.09963e4 1666.66803

000074

=====

Acq. Operator	: gxw	Seq. Line	: 11
Acq. Instrument	: LC-PDSC-06	Location	: P1-B-09
Injection Date	: 2013-10-22 2:31:13 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



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Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

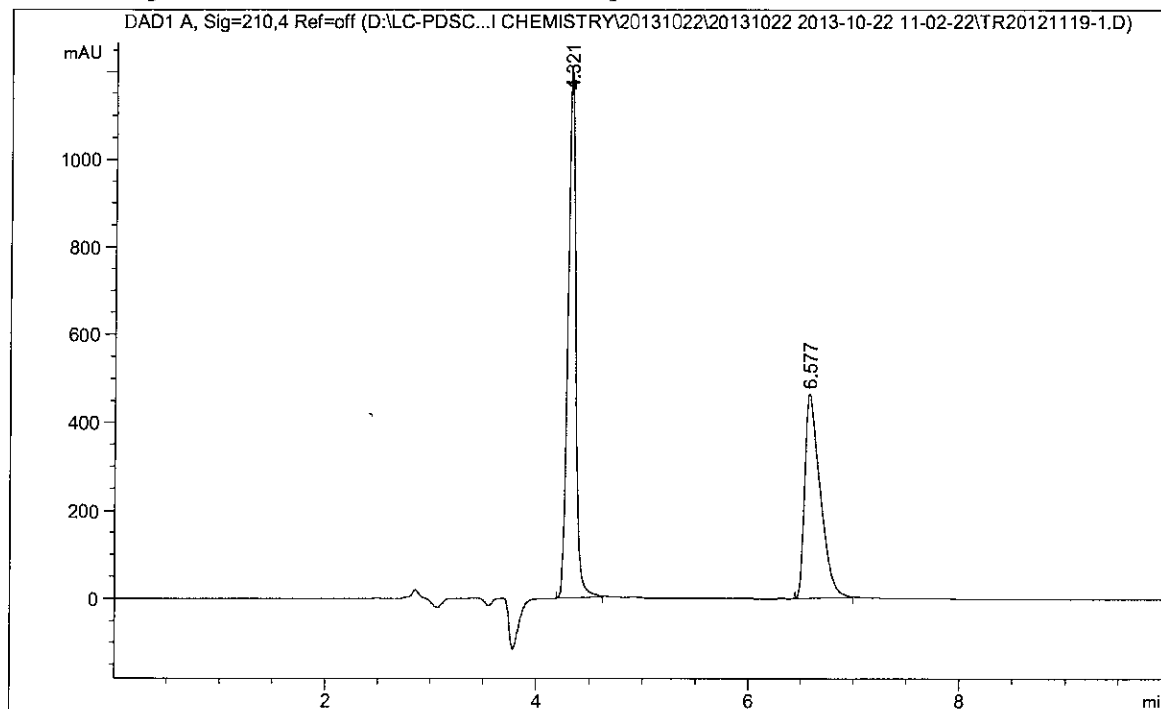
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.323	BB	0.0781	6224.21875	1207.58838	56.4445
2	6.578	BB	0.1511	4802.93799	470.31311	43.5555

Totals : 1.10272e4 1677.90149

000075

```
=====
Acq. Operator   : gxw                      Seq. Line :   12
Acq. Instrument : LC-PDSC-06              Location  : P1-C-01
Injection Date  : 2013-10-22 2:42:12 PM   Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-
                                           22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



=====
Area Percent Report
=====

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

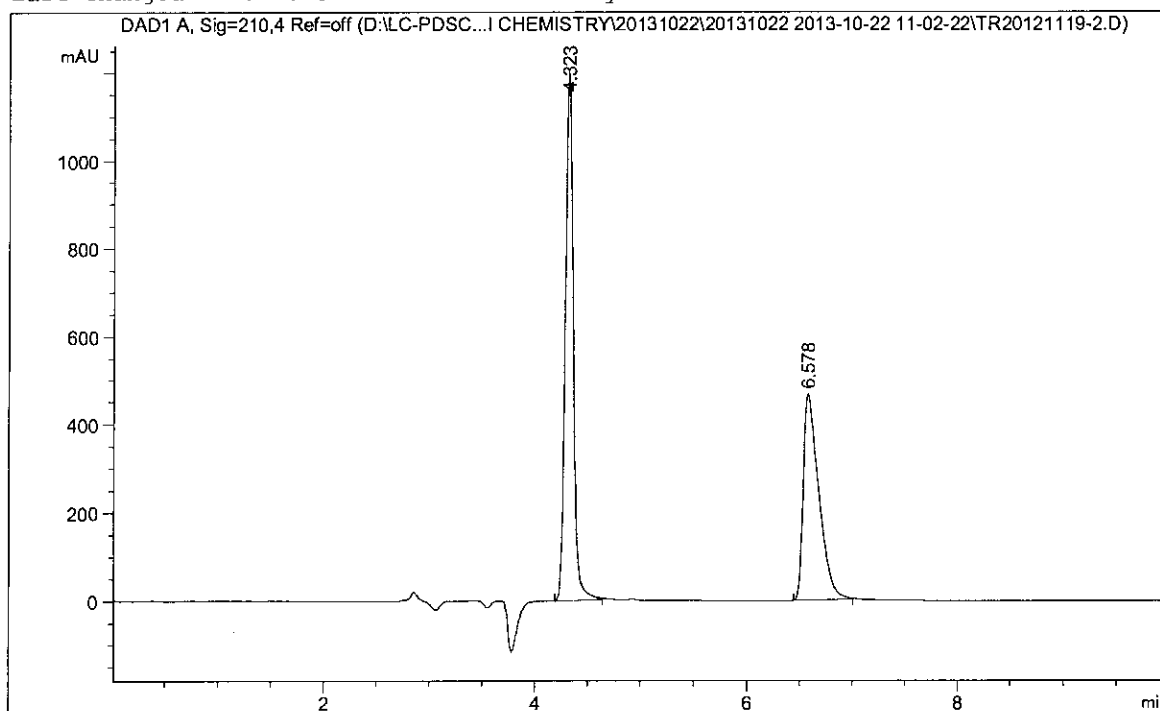
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.321	BB	0.0804	6234.22363	1204.90259	56.8295
2	6.577	BB	0.1491	4735.82568	464.08264	43.1705

Totals : 1.09700e4 1668.98523

000076

```
=====
Acq. Operator   : gxw                      Seq. Line :   13
Acq. Instrument : LC-PDSC-06              Location  : P1-C-02
Injection Date  : 2013-10-22 2:53:12 PM   Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-
                                           22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



=====
Area Percent Report
=====

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

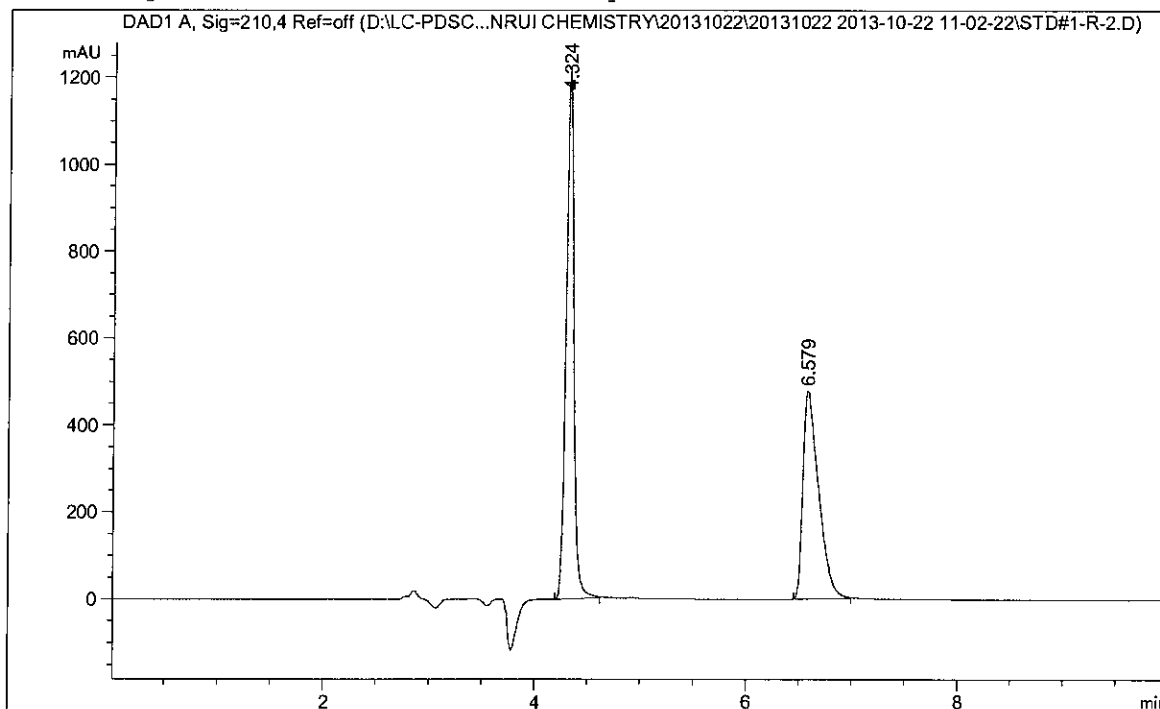
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.323	BB	0.0807	6258.43359	1202.90088	56.6034
2	6.578	BB	0.1501	4798.21289	466.14819	43.3966

Totals : 1.10566e4 1669.04907

000077

```
=====
Acq. Operator   : gxw                      Seq. Line :   14
Acq. Instrument : LC-PDSC-06              Location  : P1-B-02
Injection Date  : 2013-10-22 3:04:16 PM    Inj       :    1
                                           Inj Volume: 10 µl
Acq. Method     : D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M
Last changed    : 2013-10-22 9:42:20 AM by gxw
Analysis Method : D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M
Last changed    : 2013-10-22 4:13:23 PM by zt
=====
```



=====
Area Percent Report
=====

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 A, Sig=210,4 Ref=off

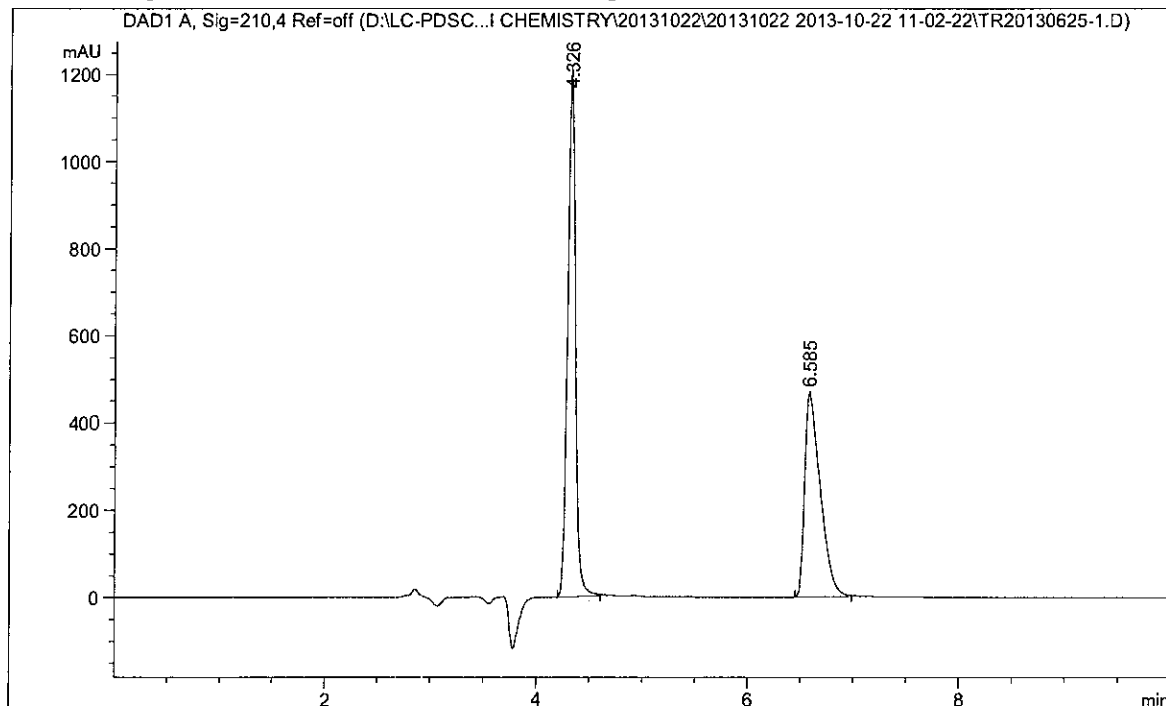
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.324	BB	0.0803	6288.06934	1216.19690	55.9961
2	6.579	BB	0.1505	4941.40479	478.46411	44.0039

Totals : 1.12295e4 1694.66101

000078

=====

Acq. Operator	: gxw	Seq. Line	: 15
Acq. Instrument	: LC-PDSC-06	Location	: P1-C-03
Injection Date	: 2013-10-22 3:15:15 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

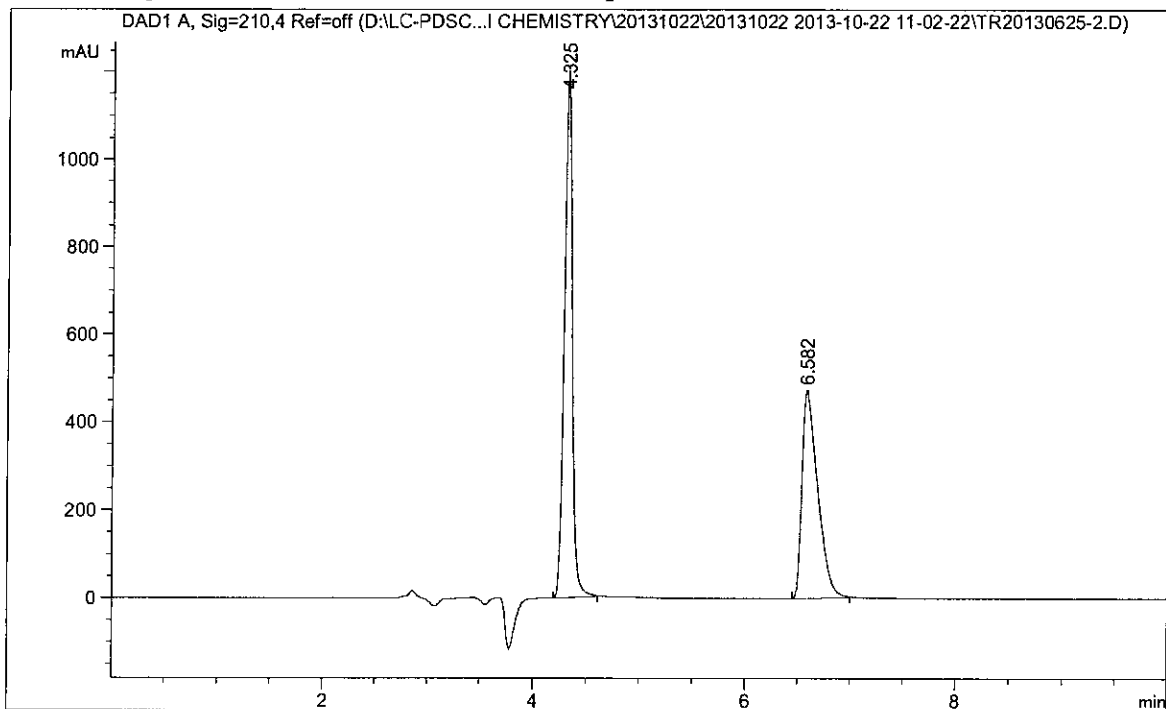
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.326	BB	0.0802	6245.01611	1210.80713	56.5172
2	6.585	BB	0.1494	4804.74219	469.56293	43.4828

Totals : 1.10498e4 1680.37006

000079

=====

Acq. Operator	: gxw	Seq. Line	: 16
Acq. Instrument	: LC-PDSC-06	Location	: P1-C-04
Injection Date	: 2013-10-22 3:26:14 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

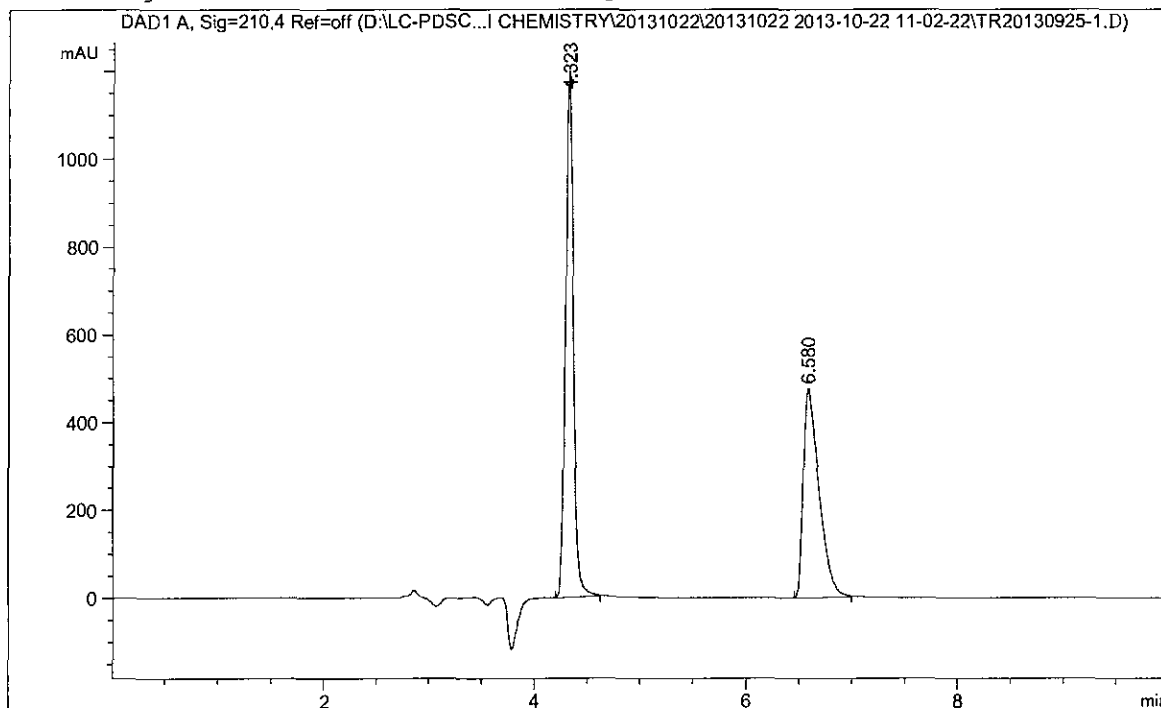
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.325	BB	0.0782	6202.29248	1201.20984	55.9491
2	6.582	BB	0.1503	4883.31494	473.74863	44.0509

Totals : 1.10856e4 1674.95847

000080

=====

Acq. Operator	: gxw	Seq. Line	: 17
Acq. Instrument	: LC-PDSC-06	Location	: P1-C-05
Injection Date	: 2013-10-22 3:37:14 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

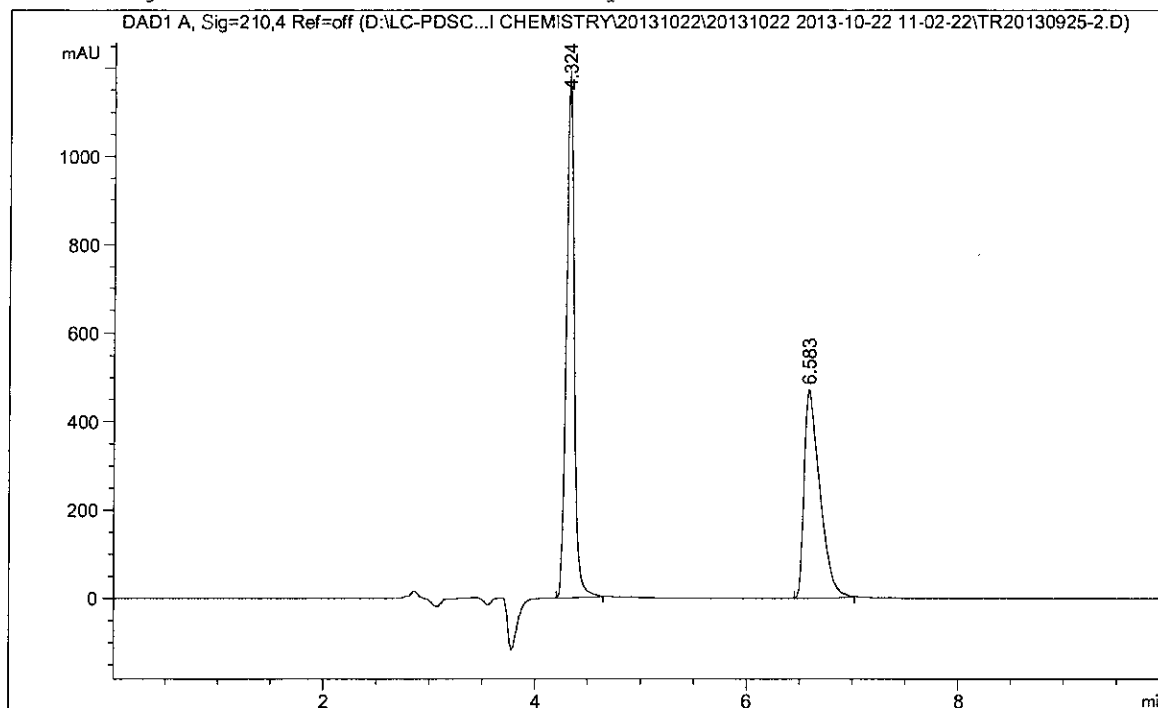
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.323	BB	0.0783	6203.75342	1200.99805	55.8539
2	6.580	BB	0.1502	4903.34570	475.82913	44.1461

Totals : 1.11071e4 1676.82718

000081

=====

Acq. Operator	: gxw	Seq. Line	: 18
Acq. Instrument	: LC-PDSC-06	Location	: P1-C-06
Injection Date	: 2013-10-22 3:48:13 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		



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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.324	BB	0.0807	6211.04199	1192.82483	56.0311
2	6.583	BB	0.1510	4873.94824	470.05017	43.9689

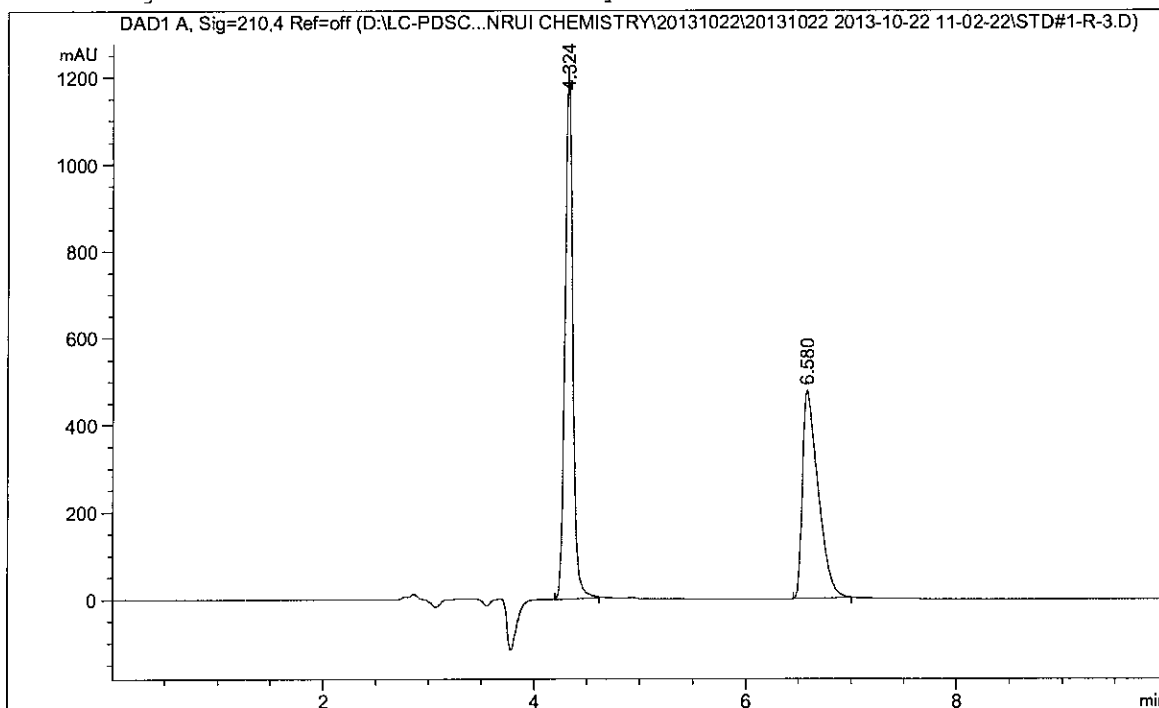
Totals : 1.10850e4 1662.87500

000082

=====

Acq. Operator	: gxw	Seq. Line	: 19
Acq. Instrument	: LC-PDSC-06	Location	: P1-B-02
Injection Date	: 2013-10-22 3:59:10 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: D:\LC-PDSC-06\tianrui chemistry\20131022\20131022 2013-10-22 11-02-22\131021.M		
Last changed	: 2013-10-22 9:42:20 AM by gxw		
Analysis Method	: D:\LC-PDSC-06\TIANRUI CHEMISTRY\131021-A.M		
Last changed	: 2013-10-22 4:13:23 PM by zt		

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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

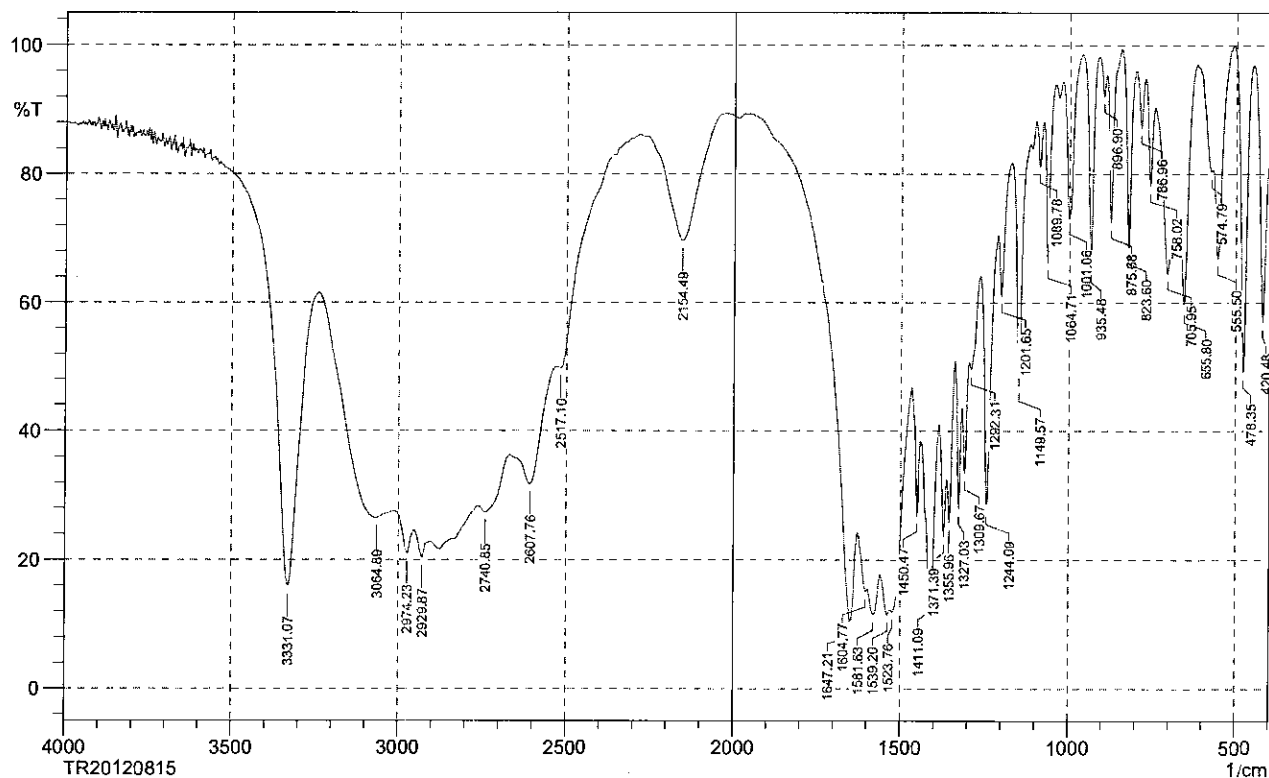
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.324	BB	0.0803	6279.04932	1215.84363	55.9488
2	6.580	BB	0.1506	4943.79687	478.13840	44.0512

Totals : 1.12228e4 1693.98203

000083

Page 80



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	420.48	57.002	31.247	449.41	401.19	5.382	3.034
2	478.35	49.131	49.246	509.21	449.41	6.014	5.599
3	555.5	66.843	18.706	570.93	509.21	4.569	1.701
4	574.79	80.363	1.937	609.51	570.93	2.208	0.109
5	655.8	59.35	20.995	673.16	619.15	5.969	1.836
6	705.95	64.529	16.092	744.52	673.16	9.002	2.415
7	758.02	78.119	14.301	773.46	744.52	1.832	0.858
8	786.96	87.272	8.198	800.46	773.46	0.979	0.436
9	823.6	68.538	29.199	846.75	800.46	2.668	2.205
10	875.68	72.485	24.22	891.11	846.75	2.109	1.585
11	896.9	92.096	3.987	912.33	891.11	0.462	0.158
12	935.48	65.556	32.894	962.48	912.33	2.747	2.41
13	1001.06	73.136	22.575	1020.34	962.48	3.319	2.401
14	1064.71	66.275	23.979	1078.21	1041.56	2.962	1.458
15	1089.78	81.127	7.03	1099.43	1078.21	1.51	0.347
16	1149.57	47.05	35.905	1172.72	1116.78	8.313	3.842
17	1201.65	60.982	12.821	1213.23	1172.72	5.777	0.946
18	1244.09	27.817	38.679	1263.37	1213.23	14.967	6.347
19	1292.31	49.57	3.479	1298.09	1265.3	8.548	0.544
20	1309.67	33.439	12.957	1317.38	1298.09	7.555	1.225
21	1327.03	27.908	19.02	1338.6	1317.38	8.828	1.895
22	1355.96	25.819	12.349	1363.67	1338.6	11.291	1.499
23	1371.39	23.61	11.67	1386.82	1363.67	11.943	1.705
24	1411.89	14.386	25.202	1440.83	1388.75	31.32	10.308
25	1450.47	26.846	14.06	1467.83	1442.75	11.043	1.6
26	1523.76	11.895	3.59	1529.55	1469.76	36.729	1.54
27	1539.2	11.432	1.493	1560.41	1535.34	21.646	0.58
28	1581.63	11.497	4.772	1597.06	1562.34	30.311	3.063
29	1604.77	15.082	2.119	1627.92	1598.99	21.374	0.57
30	1647.21	10.602	18.164	1863.24	1629.85	69.949	9.82
31	2154.49	69.624	17.887	2262.5	2034.9	22.509	9.419
32	2517.1	49.798	2.488	2528.68	2360.87	27.923	0.403
33	2607.76	31.778	10.624	2669.48	2530.61	57.928	6.686

34	2740.85	27.329	2.701	2760.14	2671.41	46.244	2.506
35	2929.87	20.372	3.014	2951.09	2916.37	22.801	0.982
36	2974.23	20.981	4.587	3014.74	2953.02	37.917	1.805
37	3064.89	26.423	8.492	3238.48	3016.67	98.228	12.611
38	3331.07	15.981	52.207	3483.44	3240.41	75.112	37.182

Comment;
TR20120815

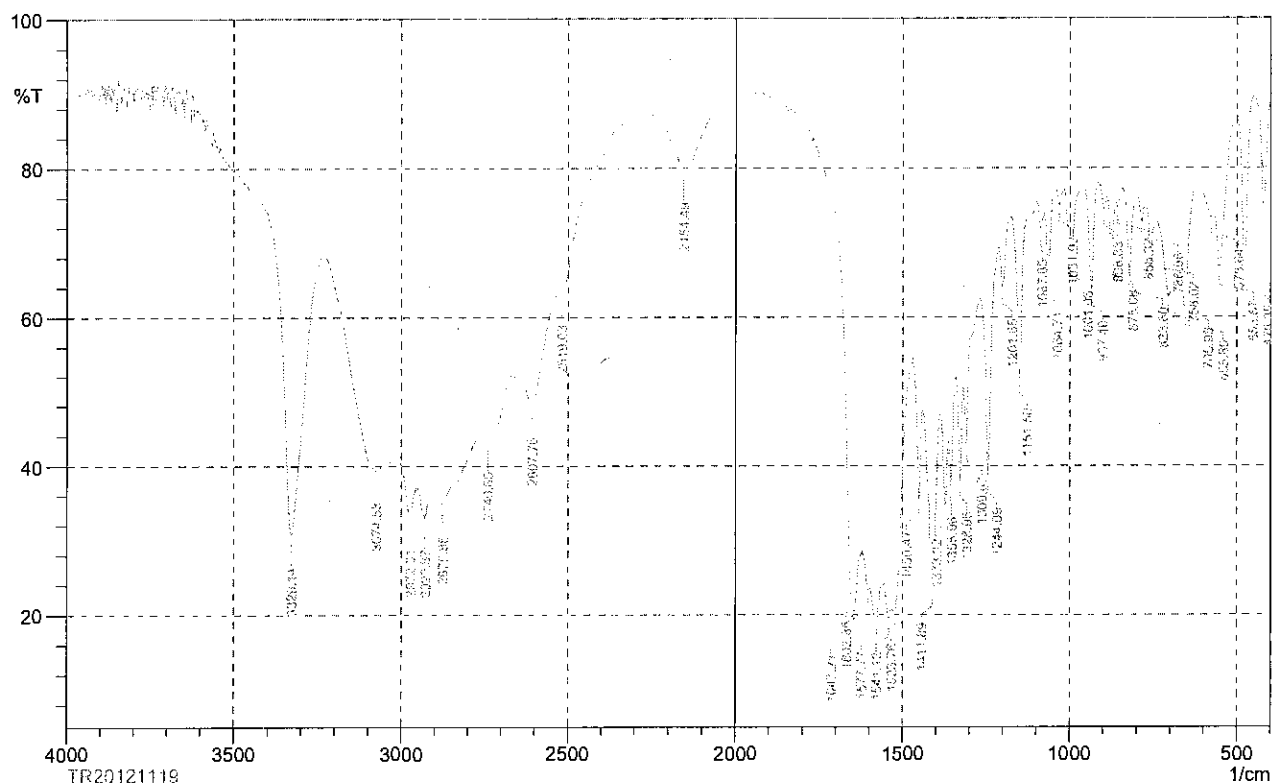
Date/Time; 10/25/2013 10:30:40 AM

No. of Scans;

Resolution;

Apodization;

User; UPLOADER



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	422.41	75.048	14.718	445.56	399.26	3.593	1.423
2	478.35	65.736	22.546	503.42	451.34	4.998	2.189
3	553.57	66.345	11.113	569	509.21	7.233	1.529
4	578.64	73.323	1.677	617.22	569	5.884	0.192
5	655.8	61.015	7.261	667.37	617.22	7.648	0.335
6	705.95	62.72	5.048	744.52	688.59	9.584	0.617
7	758.02	68.912	5.085	775.38	744.52	4.394	0.379
8	786.96	72.755	2.909	800.46	775.38	3.211	0.18
9	823.6	65.482	11.369	844.82	800.46	6.056	0.977
10	858.32	74.674	1.287	864.11	844.82	2.32	0.06
11	875.68	67.787	7.995	889.18	864.11	3.529	0.514
12	898.83	74.305	2.673	914.26	889.18	2.973	0.153
13	937.4	63.699	14.219	962.48	914.26	6.492	1.265
14	1001.06	67.672	9.572	1018.41	962.48	7.424	1.191
15	1031.92	74.947	2.258	1045.42	1018.41	3.166	0.132
16	1064.71	64.901	10.314	1076.28	1045.42	4.433	0.689
17	1087.85	71.918	2.834	1099.43	1076.28	3.104	0.178
18	1151.5	51.856	21.855	1176.58	1120.64	9.793	2.398
19	1201.65	63.93	6.893	1213.23	1178.51	5.631	0.563
20	1244.09	38.491	27.394	1271.09	1215.15	13.728	3.645
21	1309.67	42.731	10.794	1319.31	1296.16	7.031	0.834
22	1328.95	37.969	13.349	1342.46	1319.31	7.894	1.211
23	1355.96	37.318	9.205	1363.67	1342.46	7.713	0.859
24	1373.32	34.342	10.437	1386.82	1363.67	9.162	1.144
25	1411.89	23.174	23.846	1438.9	1388.75	22.99	6.559
26	1450.47	35.425	14.466	1469.76	1440.83	10.04	1.517
27	1523.76	20.372	3.605	1529.55	1471.69	25.812	0.729
28	1541.12	19.207	2.182	1554.63	1537.27	11.832	0.414
29	1577.77	19.359	4.712	1598.99	1564.27	23.282	1.769
30	1602.85	23.404	1.033	1622.13	1598.99	13.718	0.157
31	1647.21	18.976	24.122	1697.36	1622.13	34.278	8.478
32	2154.49	79.645	8.602	2250.93	2021.4	16.541	4.314
33	2519.03	63.243	0.78	2524.82	2351.23	20.576	0.109

34	2607.76	48.105	8.883	2669.48	2526.75	38.159	3.958
35	2740.85	43.457	2.482	2760.14	2671.41	29.681	1.498
36	2875.86	34.842	2.641	2900.94	2762.06	57.102	1.8
37	2929.87	33.083	3.522	2951.09	2902.87	21.99	0.891
38	2972.31	33.15	5.28	3007.02	2953.02	23.6	1.427
39	3074.53	39.359	8.535	3224.98	3022.45	63.683	7.348
40	3329.14	30.638	41.246	3425.58	3236.55	48.437	21.265

Comment;
TR20121119

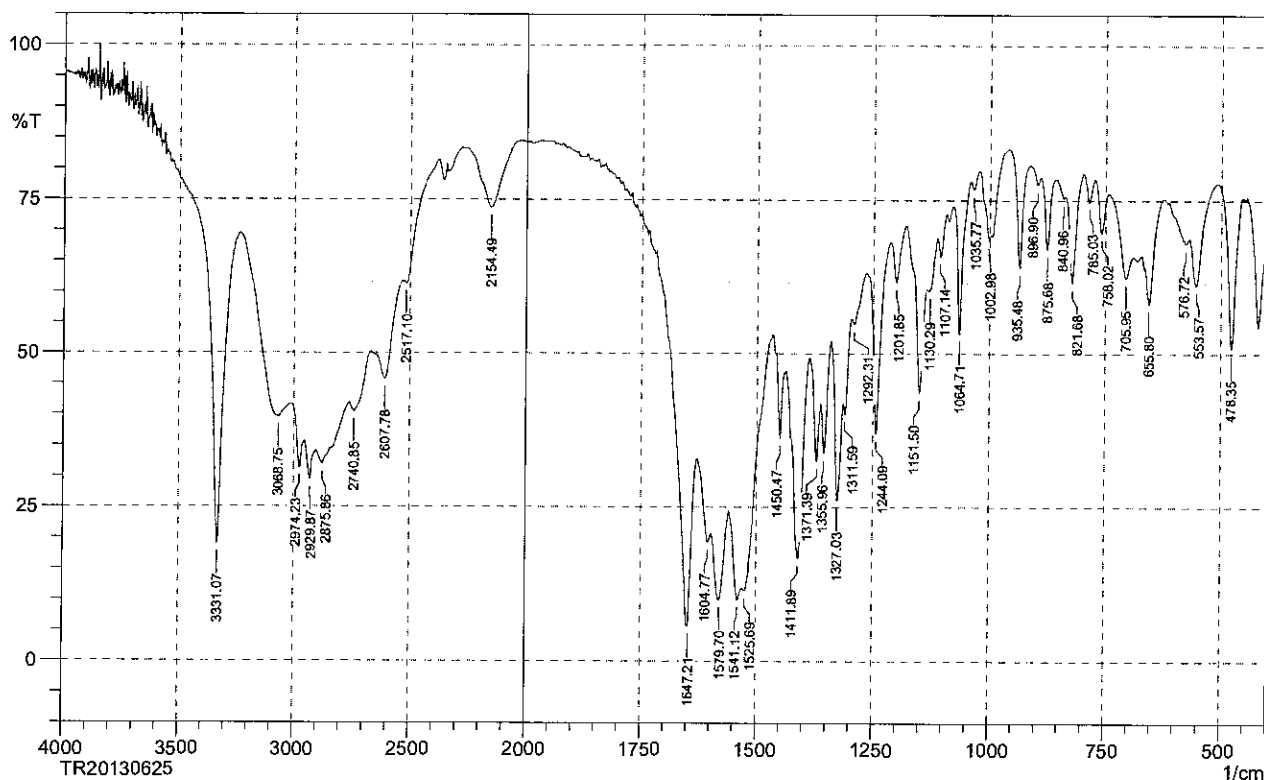
Date/Time; 10/25/2013 11:19:19 AM

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Resolution;

Apodization;

User; UPLOADER

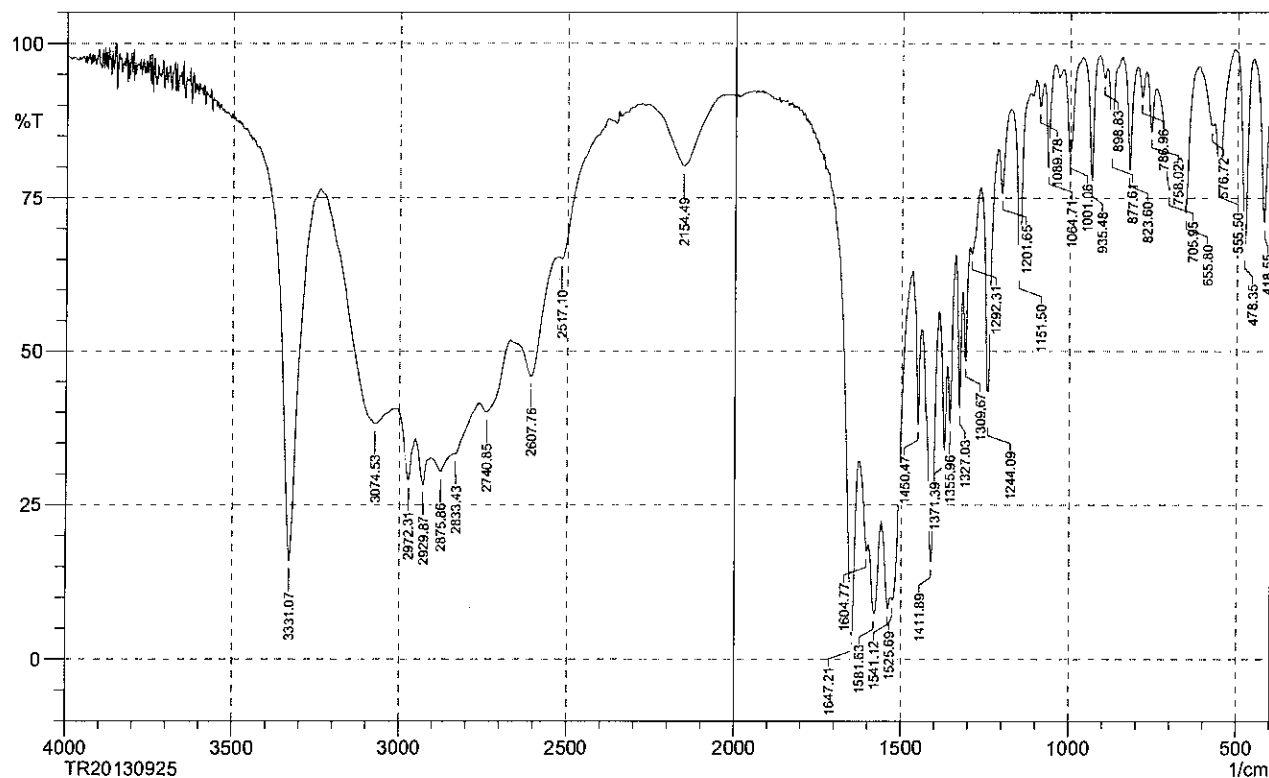


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	478.35	50.882	25.647	503.42	453.27	8.964	3.136
2	553.57	61.262	9.919	569	511.14	9.001	1.139
3	576.72	67.925	1.867	615.29	569	6.94	0.262
4	655.8	58.197	8.601	665.44	624.94	7.056	0.656
5	705.95	62.387	6.721	742.59	688.59	8.891	0.782
6	758.02	69.705	7.618	773.46	742.59	4.022	0.573
7	785.03	74.797	4.116	798.53	773.46	2.843	0.269
8	821.68	61.632	15.499	837.11	798.53	5.666	1.392
9	840.96	75.349	0.792	858.32	837.11	2.431	0.026
10	875.68	67.019	11.607	891.11	858.32	4.235	0.809
11	896.9	77.535	1.721	914.26	891.11	2.347	0.071
12	935.48	64.055	17.842	960.55	914.26	5.4	1.409
13	1002.98	68.414	3.161	1024.2	997.2	3.619	0.149
14	1035.77	76.749	2.016	1043.49	1024.2	2.08	0.098
15	1064.71	53.299	22.434	1080.14	1043.49	6.096	1.733
16	1107.14	65.867	4.149	1112.93	1093.64	3.129	0.234
17	1130.29	60.318	2.287	1136.07	1112.93	4.606	0.206
18	1151.5	43.517	20.587	1180.44	1136.07	10.623	2.396
19	1201.65	61.666	7.596	1215.15	1182.36	5.866	0.682
20	1244.09	37.001	28.237	1265.3	1215.15	12.827	3.67
21	1292.31	54.807	2.236	1298.09	1267.23	7.278	0.266
22	1311.59	40.138	4.741	1315.45	1298.09	5.837	0.34
23	1327.03	26.072	20.428	1340.53	1315.45	11.101	2.698
24	1355.96	33.835	11.338	1363.67	1340.53	8.791	1.114
25	1371.39	32.516	11.767	1386.82	1363.67	9.17	1.228
26	1411.89	16.784	31.327	1436.97	1388.75	24.452	9.124
27	1450.47	36.211	13.447	1465.9	1438.9	9.364	1.234
28	1525.69	11.492	2.945	1529.55	1467.83	32.654	0.682
29	1541.12	10.015	6.041	1560.41	1531.48	24.267	1.828
30	1579.7	10.061	12.047	1597.06	1562.34	28.782	6.047
31	1604.77	19.359	3.768	1627.92	1598.99	17.775	0.771
32	1647.21	5.724	33.772	1718.58	1629.85	45.32	15.832
33	2154.49	73.736	10.141	2258.64	2042.62	21.855	5.386

34	2517.1	61.367	2.397	2532.54	2384.02	20.822	0.397
35	2607.76	45.728	9.736	2669.48	2534.46	38.646	4.399
36	2740.85	40.479	3.179	2760.14	2671.41	31.937	1.956
37	2875.86	31.975	2.322	2902.87	2839.22	30.469	0.919
38	2929.87	29.424	5.51	2953.02	2904.8	23.545	1.476
39	2974.23	31.384	6.299	3012.81	2954.95	25.381	1.469
40	3068.75	39.579	8.864	3236.55	3014.74	67.34	7.883
41	3331.07	19.048	53.117	3446.79	3238.48	56.04	26.83

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	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	418.55	71.069	20.035	451.34	401.19	3.407	1.705
2	478.35	66.127	32.069	503.42	451.34	3.291	2.874
3	555.5	77.771	12.406	570.93	511.14	3.104	1.197
4	576.72	86.699	1.558	615.29	570.93	1.743	0.048
5	655.8	72.543	13.489	673.16	615.29	3.856	0.835
6	705.95	76.347	8.739	744.52	684.73	4.625	0.888
7	758.02	85.703	8.252	773.46	744.52	1.24	0.467
8	786.96	91.342	4.482	800.46	773.46	0.726	0.226
9	823.6	79.561	17.469	846.75	800.46	1.81	1.203
10	877.61	82.606	13.961	891.11	846.75	1.442	0.837
11	898.83	94.345	2.423	912.33	891.11	0.374	0.098
12	935.48	77.857	20.079	962.48	912.33	1.819	1.362
13	1001.06	82.454	14.041	1020.34	962.48	2.175	1.361
14	1064.71	79.861	14.906	1078.21	1043.49	1.539	0.78
15	1089.78	89.753	4.113	1099.43	1078.21	0.77	0.185
16	1151.5	62.838	27.555	1174.65	1116.78	4.833	2.366
17	1201.65	75.69	9.177	1213.23	1174.65	3.064	0.554
18	1244.09	38.837	40.572	1267.23	1213.23	9.867	4.572
19	1292.31	65.886	2.947	1298.09	1267.23	4.78	0.323
20	1309.67	48.411	14.638	1319.31	1298.09	5.198	0.981
21	1327.03	40.749	21.186	1340.53	1319.31	5.789	1.485
22	1355.96	38.245	15.378	1363.67	1340.53	7.077	1.234
23	1371.39	33.901	16.406	1388.75	1363.67	8.813	1.657
24	1411.89	15.824	39.328	1438.9	1388.75	23.485	10.495
25	1450.47	38.112	19.283	1467.83	1438.9	8.447	1.635
26	1525.69	9.624	3.85	1529.55	1469.76	29.45	0.801
27	1541.12	8.137	6.024	1560.41	1531.48	26.181	2.108
28	1581.63	7.485	12.441	1597.06	1562.34	31.708	7.549
29	1604.77	17.49	3.805	1627.92	1598.99	18.198	0.594
30	1647.21	3.891	38.23	1701.22	1629.85	39.521	17.016
31	2154.49	80.205	10.674	2260.57	2038.76	14.217	5.042
32	2517.1	65.119	1.432	2524.82	2380.16	15.312	0.164
33	2607.76	45.877	11.78	2669.48	2526.75	38.389	5.029

000090

34	2740.85	40.047	3.652	2760.14	2671.41	31.778	2.235
35	2833.43	33.278	0.225	2835.36	2762.06	31.138	0.055
36	2875.86	30.388	2.513	2902.87	2839.22	31.583	0.901
37	2929.87	28.272	5.999	2951.09	2904.8	23.208	1.548
38	2972.31	29.044	8.341	3007.02	2953.02	25.004	2.271
39	3074.53	38.234	11.207	3238.48	3020.53	64.139	9.072
40	3331.07	15.986	63.825	3421.72	3240.41	47.7	29.838

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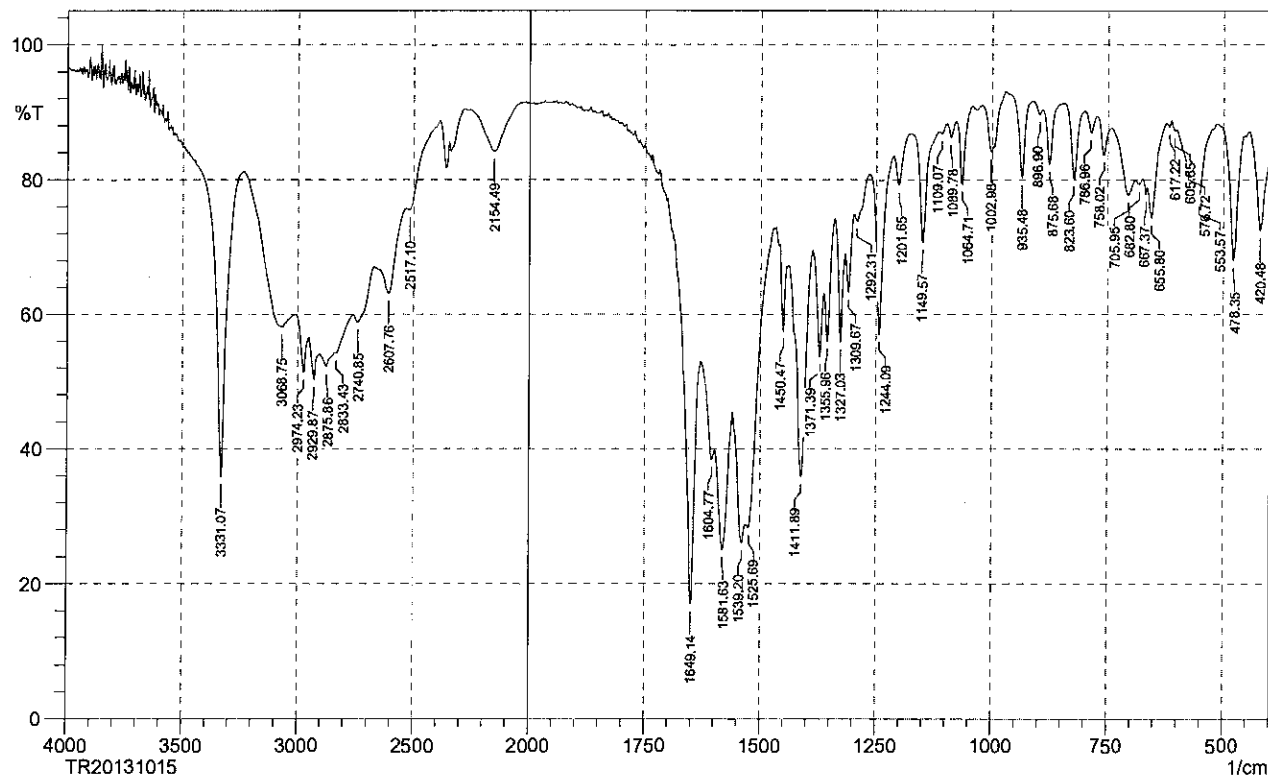
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Apodization;

User; UPLOADER

000091



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	420.48	72.421	11.914	445.56	401.19	4.334	1.117
2	478.35	68.035	19.074	503.42	457.13	4.644	1.882
3	553.57	77.172	7.372	570.93	514.99	4.494	0.696
4	576.72	82.398	1.32	601.79	570.93	2.282	0.121
5	605.65	87.267	0.61	613.36	601.79	0.655	0.019
6	617.22	87.8	0.868	624.94	613.36	0.632	0.023
7	655.8	74.335	6.829	665.44	624.94	3.638	0.468
8	667.37	77.83	1.319	675.09	665.44	0.987	0.026
9	682.8	79.33	0.705	690.52	675.09	1.523	0.031
10	705.95	77.755	4.534	742.59	690.52	4.464	0.463
11	758.02	83.701	4.897	773.46	742.59	1.919	0.296
12	786.96	87.075	2.744	800.46	773.46	1.41	0.151
13	823.6	80.181	10.584	846.75	800.46	2.71	0.762
14	875.68	82.367	8.364	891.11	846.75	2.346	0.501
15	896.9	89.696	1.056	912.33	891.11	0.911	0.045
16	935.48	80.319	11.916	970.19	912.33	2.83	0.837
17	1002.98	84.152	2.273	1020.34	997.2	1.356	0.059
18	1064.71	79.363	10.146	1078.21	1043.49	2.213	0.581
19	1089.78	86.312	2.133	1097.5	1078.21	1.124	0.098
20	1109.07	86.904	0.623	1114.86	1097.5	1.013	0.025
21	1149.57	70.747	16.209	1178.51	1114.86	5.308	1.449
22	1201.65	79.249	5.347	1213.23	1178.51	2.776	0.349
23	1244.09	57.046	24.855	1265.3	1213.23	6.82	2.37
24	1292.31	73.832	2.074	1298.09	1265.3	3.781	0.202
25	1309.67	63.308	8.064	1317.38	1298.09	3.176	0.412
26	1327.03	55.931	15.276	1340.53	1317.38	4.27	0.911
27	1355.96	56.044	10.73	1363.67	1340.53	4.516	0.7
28	1371.39	53.668	11.538	1386.82	1363.67	4.949	0.804
29	1411.89	35.865	32.565	1438.9	1386.82	14.163	5.566
30	1450.47	57.508	11.275	1458.18	1442.75	3.095	0.585
31	1525.69	28.274	3.47	1529.55	1473.62	16.815	0.428
32	1539.2	26.033	6.385	1558.48	1533.41	12.366	1.022
33	1581.63	25.112	17.005	1597.06	1560.41	17.657	4.081

000092

34	1604.77	38.406	4.061	1627.92	1598.99	10.157	0.359
35	1649.14	17.071	41.951	1716.65	1629.85	24.748	8.711
36	2154.49	84.189	6.679	2256.71	2042.62	12.013	3.135
37	2517.1	75.536	1.578	2532.54	2387.87	11.734	0.216
38	2607.76	63.173	7.871	2669.48	2534.46	22.502	2.713
39	2740.85	58.885	2.651	2760.14	2671.41	18.618	1.142
40	2833.43	54.239	0.489	2839.22	2762.06	18.748	0.087
41	2875.86	52.165	2.027	2902.87	2841.15	16.87	0.454
42	2929.87	50.243	5.2	2951.09	2904.8	12.71	0.792
43	2974.23	51.313	6.533	3010.88	2953.02	14.617	1.042
44	3068.75	58.134	0.226	3072.6	3012.81	13.65	0.027
45	3331.07	35.869	46.112	3446.79	3238.48	33.789	15.902

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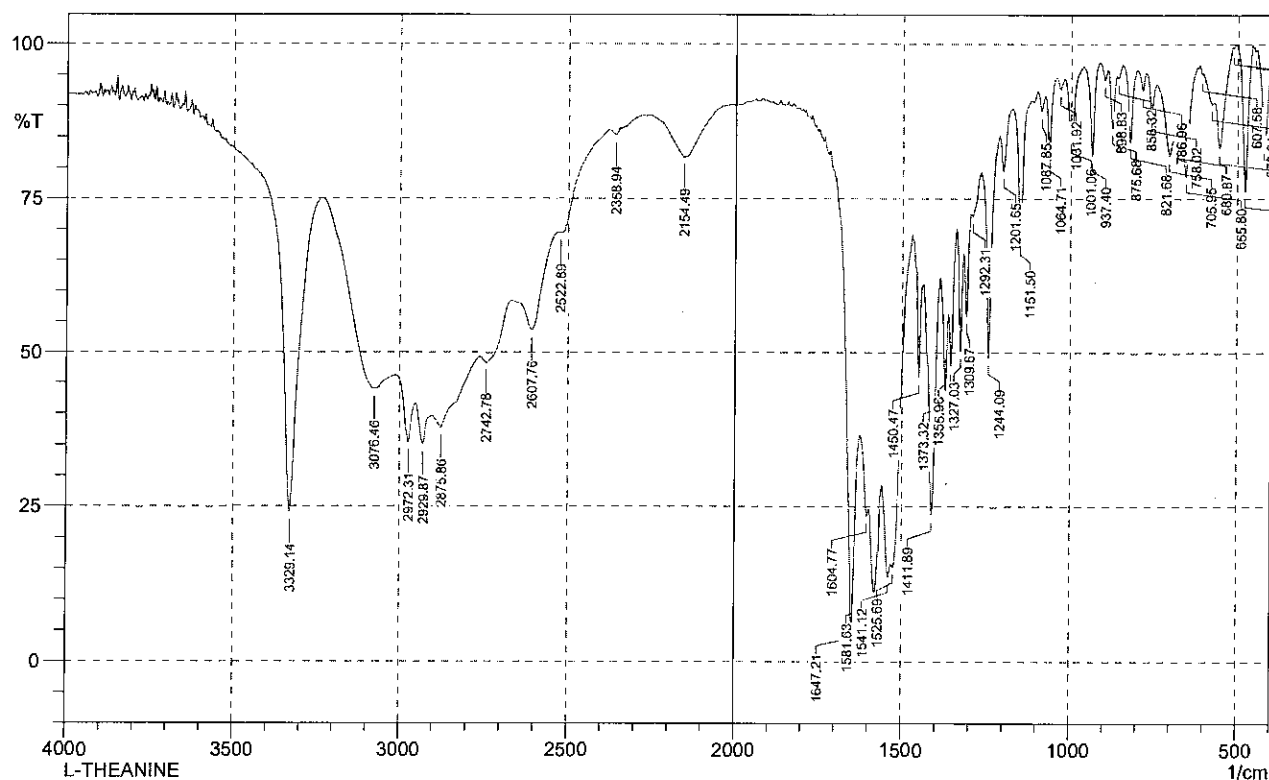
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User; UPLOADER



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	422.41	84.049	15.094	443.63	399.26	1.666	1.5
2	447.49	98.744	0.623	451.34	443.63	0.032	0.011
3	478.35	76.234	23.716	503.42	457.13	2.043	2.034
4	513.07	99.355	0.356	516.92	507.28	0.016	0.006
5	555.5	83.323	9.609	569	516.92	2.237	1.07
6	578.64	90.332	1.59	603.72	569	1.235	0.13
7	607.58	95.078	0.649	615.29	603.72	0.222	0.014
8	655.8	78.667	9.775	673.16	621.08	2.993	0.663
9	680.87	84.239	0.426	688.59	673.16	1.134	0.018
10	705.95	82.083	5.533	742.59	688.59	3.226	0.511
11	758.02	88.568	5.818	775.38	742.59	1.142	0.327
12	786.96	92.497	3.116	800.46	775.38	0.636	0.15
13	821.68	84.207	12.114	844.82	800.46	1.542	0.823
14	858.32	94.653	0.831	864.11	844.82	0.385	0.029
15	875.68	86.529	8.8	891.11	864.11	1.007	0.454
16	898.83	94.275	1.97	914.26	891.11	0.448	0.084
17	937.4	82.138	14.612	964.41	914.26	1.722	0.997
18	1001.06	85.141	10.017	1020.34	964.41	2.1	0.968
19	1031.92	92.726	1.854	1043.49	1020.34	0.658	0.098
20	1064.71	82.219	10.56	1078.21	1043.49	1.636	0.559
21	1087.85	89.227	2.719	1099.43	1078.21	0.9	0.129
22	1151.5	68.537	21.6	1176.58	1118.71	4.396	1.811
23	1201.65	79.485	7.104	1213.23	1176.58	2.601	0.45
24	1244.09	49.043	32.797	1267.23	1213.23	7.877	3.282
25	1292.31	72.153	1.275	1296.16	1267.23	3.572	0.098
26	1309.67	55.917	12.812	1319.31	1296.16	4.458	0.757
27	1327.03	50.269	17.24	1340.53	1319.31	4.654	1.106
28	1355.96	47.826	13.085	1363.67	1340.53	5.595	0.924
29	1373.32	43.8	14.942	1386.82	1363.67	6.556	1.279
30	1411.89	23.637	37.994	1436.97	1388.75	17.997	7.86
31	1450.47	45.891	18.334	1471.69	1436.97	7.852	1.353
32	1525.69	15.286	5.757	1531.48	1473.62	24.508	0.989
33	1541.12	13.677	5.872	1558.48	1533.41	18.691	1.479

34	1581.63	11.281	14.849	1597.06	1560.41	27.522	6.375
35	1604.77	23.454	3.887	1624.06	1598.99	13.653	0.489
36	1647.21	6.348	40.967	1716.65	1625.99	36.12	13.74
37	2154.49	81.743	7.426	2252.86	2040.69	14.086	3.583
38	2358.94	85.33	0.986	2380.16	2345.44	2.311	0.087
39	2522.89	69.421	0.709	2528.68	2380.16	15.09	0.168
40	2607.76	53.679	9.554	2667.55	2530.61	30.401	3.679
41	2742.78	48.262	2.786	2760.14	2669.48	25.929	1.521
42	2875.86	37.707	3.647	2900.94	2762.06	51.286	2.158
43	2929.87	35.014	5.696	2951.09	2902.87	20.172	1.265
44	2972.31	35.442	7.648	3012.81	2953.02	23.16	1.747
45	3076.46	43.936	10.386	3234.62	3014.74	58.118	7.76
46	3329.14	24.081	53.469	3446.79	3236.55	46.123	23.205

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Resolution;
Apodization;
User; UPLOADER

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APPENDIX B

MICROBIOLOGICAL AND HEAVY METALS ANALYSIS OF MULTIPLE LOTS OF TIANRUI CHEMICAL'S L-THEANINE INGREDIENT

Lot Numbers: TR20130625

TR20130925

TR20131015

TR20120815

TR20121119

000096

**Test Report****Report No: SHAFFG131007916-1****Date: Oct 30 2013**

Client name: Zhejiang Tianrui Chemical Co., Ltd.
Client address: No.12 Xingye Road, South Industrial Zone, Longyou County, Zhejiang Province, China
Sample name: L-Theanine
Sample Batch No.: TR20130625
Product Date: 20130625
Manufacturer: Zhejiang Tianrui Chemical Co., Ltd.

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Sample No.: SHAFFG131007916-1
SGS reference No.: HZAFO131001124-1 /SHAFD1320835501
Date of sample received: Oct 24 2013
Testing period: Oct 24 2013 ~ Oct 30 2013

TEST(S) REQUESTED:

Selected test(s) as requested by the applicant

TEST METHOD(S):

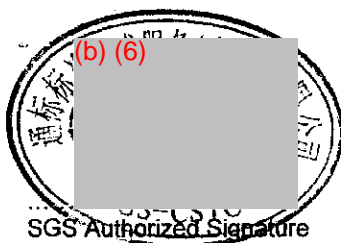
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TEST RESULT(S):

Please refer to next page(s)

SAMPLE DESCRIPTION: Powder in bag

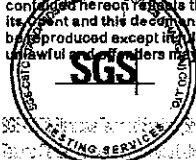
Unless otherwise stated the results shown in this test report refer only to the sample(s) tested. This document cannot be used for publicity, without prior written approval of the SGS.



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Page 1 of 2

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e sgs.china@sgs.com

039604

Member of the SGS Group (SGS SA)

Test Report

Report No: SHAFFG131007916-1

Date: Oct 30 2013

TEST METHOD(S):

Aerobic plate count: GB 4789.2-2010 National food safety standard Food Microbiological examination:

Aerobic plate count

Mould & Yeast: GB 4789.15-2010 National food safety standard Food microbiological examination:

Enumeration of moulds and yeasts

Salmonella: GB 4789.4-2010 National food safety standard Food microbiological examination:

Salmonella

Mercury: GB/T 5009.17-2003 Determination of total mercury and organic-mercury in foods I

Lead: GB 5009.12-2010 National food safety standard Determination of lead in foods I

Cadmium: GB/T 5009.15-2003 Determination of cadmium in foods I

Chromium: GB/T 5009.123-2003 Determination of chromium in foods

Arsenic: GB/T 5009.11-2003 Determination of total arsenic and Inorganic arsenic in foods I

TEST RESULT(S):

Chemical test

Test item(s)	Unit(s)	Test method(s)	Test result(s)	Method detection limit(s)
Mercury	mg/kg	GB/T 5009.17-2003 I	Not detected	0.001
Lead	mg/kg	GB 5009.12-2010 I	0.034	0.005
Cadmium	mg/kg	GB/T 5009.15-2003 I	Not detected	0.005
Chromium	mg/kg	GB/T 5009.123-2003 I	Not detected	0.1
Arsenic	mg/kg	GB/T 5009.11-2003 I	Not detected	0.01

Microbe test

Test item(s)	Unit(s)	Test method(s)	Test result(s)
Aerobic plate count	cfu/g	GB 4789.2-2010	< 10
Mould & Yeast	cfu/g	GB 4789.15-2010	< 10
Salmonella /25g	--	GB 4789.4-2010	Not detected

*** End of Report***

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Member of the SGS Group (SGS SA)

**Testing Summary**

Summary No: SHAFFG131007916T-1

Issue Date: Oct 30 2013

Client name: Zhejiang Tianrui Chemical Co., Ltd.
Client address: No.12 Xingye Road, South Industrial Zone, Longyou County, Zhejiang Province, China
Sample name: L-Theanine
Sample Batch No.: TR20130625
Product Date: 20130625
Manufacturer: Zhejiang Tianrui Chemical Co., Ltd.

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Sample No.: SHAFFG131007916-1
SGS reference No.: HZAFO131001124-1
Date of sample received: Oct 24 2013
Testing period: Oct 24 2013 ~ Oct 30 2013

TEST(S) REQUESTED:

Selected test(s) as requested by applicant

TEST METHOD(S):

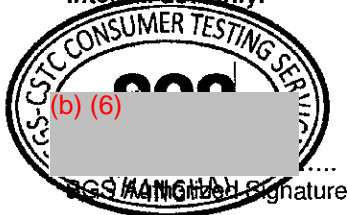
Escherichia: ISO 7251-2005 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli --Most probable number technique

TEST RESULT(S):

Test item(s)	Unit(s)	Test method(s)	Test result(s)
Escherichia /25g	--	ISO 7251-2005	Not detected

SAMPLE DESCRIPTION: Powder in bag

The results shown in this test summary refer only to the sample(s) tested, and for clients internal use only.



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000099

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F2011090015

TESTING
CNAS L0599**Test Report****Report No: SHAFFG131007916-2****Date: Oct 30 2013**

Client name: Zhejiang Tianrui Chemical Co., Ltd.
Client address: No.12 Xingye Road, South Industrial Zone, Longyou County, Zhejiang Province, China
Sample name: L-Theanine
Sample Batch No.: TR20130925
Product Date: 20130925
Manufacturer: Zhejiang Tianrui Chemical Co., Ltd.

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Sample No.: SHAFFG131007916-2
SGS reference No.: HZAFO131001124-2 /SHAFO1320835501
Date of sample received: Oct 24 2013
Testing period: Oct 24 2013 ~ Oct 30 2013

TEST(S) REQUESTED:

Selected test(s) as requested by the applicant

TEST METHOD(S):

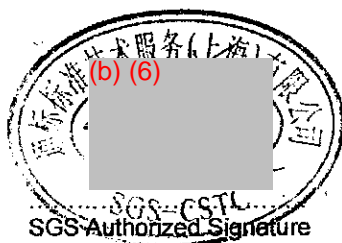
Please refer to next page(s)

TEST RESULT(S):

Please refer to next page(s)

SAMPLE DESCRIPTION: Powder in bag

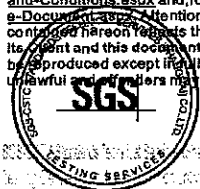
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Test Report

Report No: SHAFFG131007916-2

Date: Oct 30 2013

TEST METHOD(S):

Aerobic plate count: GB 4789.2-2010 National food safety standard Food Microbiological examination:

Aerobic plate count

Mould & Yeast: GB 4789.15-2010 National food safety standard Food microbiological examination:

Enumeration of moulds and yeasts

Salmonella: GB 4789.4-2010 National food safety standard Food microbiological examination:

Salmonella

Mercury: GB/T 5009.17-2003 Determination of total mercury and organic-mercury in foods I

Lead: GB 5009.12-2010 National food safety standard Determination of lead in foods I

Cadmium: GB/T 5009.15-2003 Determination of cadmium in foods I

Chromium: GB/T 5009.123-2003 Determination of chromium in foods

Arsenic: GB/T 5009.11-2003 Determination of total arsenic and Inorganic arsenic in foods I

TEST RESULT(S):

Chemical test

Test item(s)	Unit(s)	Test method(s)	Test result(s)	Method detection limit(s)
Mercury	mg/kg	GB/T 5009.17-2003 I	Not detected	0.001
Lead	mg/kg	GB 5009.12-2010 I	Not detected	0.005
Cadmium	mg/kg	GB/T 5009.15-2003 I	Not detected	0.005
Chromium	mg/kg	GB/T 5009.123-2003 I	0.1	0.1
Arsenic	mg/kg	GB/T 5009.11-2003 I	Not detected	0.01

Microbe test

Test item(s)	Unit(s)	Test method(s)	Test result(s)
Aerobic plate count	cfu/g	GB 4789.2-2010	< 10
Mould & Yeast	cfu/g	GB 4789.15-2010	< 10
Salmonella /25g	—	GB 4789.4-2010	Not detected

*** End of Report***

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SHAFF 039607

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**Testing Summary****Summary No: SHAFFG131007916T-2****Issue Date: Oct 30 2013**

Client name: Zhejiang Tianrui Chemical Co., Ltd.
Client address: No.12 Xingye Road, South Industrial Zone, Longyou County, Zhejiang Province, China
Sample name: L-Theanine
Sample Batch No.: TR20130925
Product Date: 20130925
Manufacturer: Zhejiang Tianrui Chemical Co., Ltd.

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Sample No.: SHAFFG131007916-2
SGS reference No.: HZAFO131001124-2
Date of sample received: Oct 24 2013
Testing period: Oct 24 2013 ~ Oct 30 2013

TEST(S) REQUESTED:

Selected test(s) as requested by applicant

TEST METHOD(S):

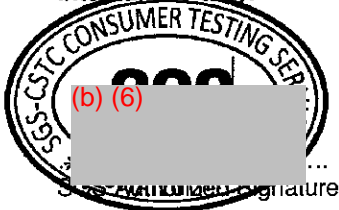
Escherichia: ISO 7251-2005 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli --Most probable number technique

TEST RESULT(S):

Test item(s)	Unit(s)	Test method(s)	Test result(s)
Escherichia /25g	--	ISO 7251-2005	Not detected

SAMPLE DESCRIPTION: Powder in bag

The results shown in this test summary refer only to the sample(s) tested, and for clients internal use only.



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000102

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F2011090015

TESTING
CNAS L0599

Test Report

Report No: SHAFFG131007916-3

Date: Oct 30 2013

Client name: Zhejiang Tianrui Chemical Co., Ltd.
Client address: No.12 Xingye Road, South Industrial Zone, Longyou County, Zhejiang Province, China
Sample name: L-Theanine
Sample Batch No.: TR20131015
Product Date: 20131015
Manufacturer: Zhejiang Tianrui Chemical Co., Ltd.

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Sample No.: SHAFFG131007916-3
SGS reference No.: HZAFO131001124-3 / SHAFD1320835501
Date of sample received: Oct 24 2013
Testing period: Oct 24 2013 ~ Oct 30 2013

TEST(S) REQUESTED:

Selected test(s) as requested by the applicant

TEST METHOD(S):

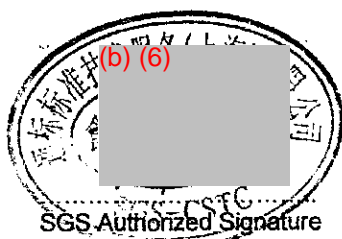
Please refer to next page(s)

TEST RESULT(S):

Please refer to next page(s)

SAMPLE DESCRIPTION: Powder in bag

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Page 100

Test Report

Report No: SHAFFG131007916-3

Date: Oct 30 2013

TEST METHOD(S):

Aerobic plate count: GB 4789.2-2010 National food safety standard Food Microbiological examination:

Aerobic plate count

Mould & Yeast: GB 4789.15-2010 National food safety standard Food microbiological examination:

Enumeration of moulds and yeasts

Salmonella: GB 4789.4-2010 National food safety standard Food microbiological examination:

Salmonella

Mercury: GB/T 5009.17-2003 Determination of total mercury and organic-mercury in foods I

Lead: GB 5009.12-2010 National food safety standard Determination of lead in foods I

Cadmium: GB/T 5009.15-2003 Determination of cadmium in foods I

Chromium: GB/T 5009.123-2003 Determination of chromium in foods

Arsenic: GB/T 5009.11-2003 Determination of total arsenic and Inorganic arsenic in foods I

TEST RESULT(S):

Chemical test

Test item(s)	Unit(s)	Test method(s)	Test result(s)	Method detection limit(s)
Mercury	mg/kg	GB/T 5009.17-2003 I	Not detected	0.001
Lead	mg/kg	GB 5009.12-2010 I	Not detected	0.005
Cadmium	mg/kg	GB/T 5009.15-2003 I	Not detected	0.005
Chromium	mg/kg	GB/T 5009.123-2003 I	Not detected	0.1
Arsenic	mg/kg	GB/T 5009.11-2003 I	Not detected	0.01

Microbe test

Test item(s)	Unit(s)	Test method(s)	Test result(s)
Aerobic plate count	cfu/g	GB 4789.2-2010	< 10
Mould & Yeast	cfu/g	GB 4789.15-2010	M: 10, Y: < 10
Salmonella /25g	—	GB 4789.4-2010	Not detected

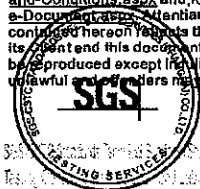
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**Testing Summary**

Summary No: SHAFFG131007916T-3

Issue Date: Oct 30 2013

Client name: Zhejiang Tianrui Chemical Co., Ltd.
Client address: No.12 Xingye Road, South Industrial Zone, Longyou County, Zhejiang Province, China
Sample name: L-Theanine
Sample Batch No.: TR20131015
Product Date: 20131015
Manufacturer: Zhejiang Tianrui Chemical Co., Ltd.

Above information and sample(s) was/were submitted and certified by the client, SGS quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

SGS Sample No.: SHAFFG131007916-3
SGS reference No.: HZAFO131001124-3
Date of sample received: Oct 24 2013
Testing period: Oct 24 2013 ~ Oct 30 2013

TEST(S) REQUESTED:

Selected test(s) as requested by applicant

TEST METHOD(S):

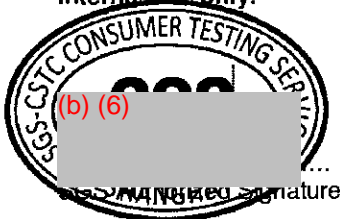
Escherichia: ISO 7251-2005 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli --Most probable number technique

TEST RESULT(S):

Test item(s)	Unit(s)	Test method(s)	Test result(s)
Escherichia /25g	--	ISO 7251-2005	Not detected

SAMPLE DESCRIPTION: Powder in bag

The results shown in this test summary refer only to the sample(s) tested, and for clients internal use only.



*** End ***

000105

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AFFILIATE CODE: F615001

Report NO. : AGLSHW2012081570(1)

Report Date : 2012.09.04

Page 1 of 1

TEST REPORT

Applicant : Zhejiang Tianrui Chemical CO.,Ltd
Sample described as : L-theanine
Lot No.: TR20120815
Date of sample received : 2012.08.27
Testing date : 2012.08.27-2012.09.04

We report that in pursuance of an order received from the applicant as above, the submitted sample was tested for requested analysis in SGS Shanghai laboratory and we certify the results as follows:

Sample description: powder in bag

TEST RESULT(S):

Test Items	Test Methods	MDL	Test Results
Mercury (Hg) mg/kg	With reference to GB/T5009-2003, analysis was performed by ICP-MS	0.005	Not detected
Chromium (Cr) mg/kg	With reference to GB/T5009-2003, analysis was performed by ICP-MS	0.1	Not detected
Cadmium (Cd) mg/kg	With reference to GB/T5009-2003, analysis was performed by ICP-MS	0.005	Not detected
Arsenic (As) mg/kg	With reference to GB/T5009-2003, analysis was performed by ICP-MS	0.005	Not detected
Lead (Pb) mg/kg	With reference to GB 5009.12-2010	0.005	0.007
Ethanol mg/kg	With reference to US EPA 5021A:2003, US EPA 8260C:2006, analysis was performed by HS-GC-MS	5	Not detected
Total Plate Count CFU/g	GB 4789.2-2010	-	<10
Coliforms MPN/g	GB 4789.3-2010 I	-	<0.3
Mould CFU/g	GB 4789.15-2010	-	<10
Yeast CFU/g	GB 4789.15-2010	-	<10
Salmonella spp. /25g	GB 4789.4-2010		Not detected

*** End of Report***

This analysis report reflects our findings on the sample submitted by the client only.

The content of this report does not evidence / verify shipment or refer to any consignment or other matters.

Shanghai, September 04, 2012

Signed for and on behalf of
SGS-CSTC Standards Technical Service (Shanghai) Co., Ltd.

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000106



F2011090015

AFFILIATE CODE: F615001

Report NO. : AGLSHW2012120005(1)-1

Report Date : 2012.12.12

Page 1 of 1

TEST REPORT

Applicant : Zhejiang Tianrui Chemical CO.,Ltd.

Sample ID : L-theanine
Batch no:TR20121119

Date of sample received : 2012.12.03

Testing date : 2012.12.03-2012.12.12

We report that in pursuance of an order received from the applicant as above, the submitted sample was tested for requested analysis in SGS shanghai laboratory and we certify the results as follows:

Sample description : Powder in bag

TEST RESULT(S):

Test item(s)	CAS NO	Test method(s)	Test result(s)	MDL
Aerobic plate count cfu/g	-	GB 4789.2-2010	< 10	-
Escherichia coli /25g	-	ISO 7251:2005	Not detected	-
Mould & Yeast cfu/g	-	GB 4789.15-2010	< 10	-
Salmonella /25g	-	GB 4789.4-2010	Not detected	-
Mercury mg/kg	-	GB/T 5009.17-2003 I	0.002	0.001
Lead(Pb) mg/kg	-	With reference to GB 5009.12-2010	0.010	0.005
Cadmium (Cd) mg/kg	-	GB/T 5009.15-2003	Not detected	0.005
Chromium (Cr) mg/kg	-	GB/T 5009.123-2003	0.2	0.1
Arsenic (As) mg/kg	-	GB/T 5009.11-2003	0.009	0.005
Ethanol mg/kg	64-17-5	With reference to US EPA 5021A:2003, analysis was performed by HS-GC-MS	Not detected	5

Remark: MDL=Method Detection Limit

*** End of Report***

This analysis report reflects our findings on the sample submitted by the client only.

The content of this report does not evidence / verify shipment or refer to any consignment or other matters.

Signed for and on behalf of

Shanghai, December 12, 2012

SGS-CSTC Standards Technical Service (Shanghai) Co., Ltd.

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000107

APPENDIX C

SIGNED EXPERT OPINION STATEMENT REGARDING THE GRAS STATUS OF TIANRUI CHEMICAL'S L-THEANINE INGREDIENT

000108



Expert Opinion Statement Regarding the GRAS Status of L-Theanine

Zhejiang Tianrui Chemical Co., Ltd. (Zhejiang Province, China), also referred to as Tianrui Chemical, wishes to use its manufactured ingredient, *L-theanine* ($\geq 98\%$), a high-purity chemically-synthesized L-theanine, in human foods marketed in the United States. As such, Tianrui Chemical has reviewed available information supporting a possible conclusion that *L-theanine* ($\geq 98\%$) would be generally recognized as safe (GRAS) through scientific procedures, in accordance with Section 201(s) of the federal food, Drug, and Cosmetic Act.

To support a GRAS determination, Tianrui Chemical compiled information about L-theanine, both published and unpublished, into a dossier (GRAS dossier). It is important to note, however, that Tianrui relied primarily on two prior GRAS notices filed by U.S. FDA with no objections as the basis for the conclusion that the use of their specific L-theanine ingredient [*L-theanine* ($\geq 98\%$)] is GRAS.

GRAS notice GRN No. 209, submitted by Taiyo International, Inc., asserted that the use of L-theanine (Suntheanine®, $\geq 98\%$ purity) derived enzymatically (glutaminase from *Pseudomonas nitroreducens* or *Bacillus amyloliquefaciens*) as an ingredient in fruit juices and drinks, non-herbal teas, sports beverages, bottled waters, chocolate bars and chews, hard candies, breath mints, and chewing gum at levels up to 250 mg per serving was GRAS; GRN No. 338, submitted by Blue California, made the same conclusion about L-theanine (L-TeaActive™; $\geq 98\%$ purity) derived through aqueous extraction of tea leaves, used in the same foods and at the same levels. In both cases, FDA indicated based on the available information that it had no questions regarding each company's conclusion that L-theanine is GRAS under the intended conditions of use.

Tianrui Chemical's L-theanine ingredient is substantially equivalent to these two substances and is intended for the same food applications (i.e., fruit juices and drinks, non-herbal teas, sports beverages, bottled waters, chocolate bars and chews, hard candies, breath mints, and chewing gum at levels up to 250 mg per serving); it differs only in that it is synthesized chemically from L-glutamic acid and ethylamine. Tianrui Chemical has indicated that *L-theanine* ($\geq 98\%$) is manufactured from food-grade materials in accordance with current Good Manufacturing Practices (cGMP), and that quality is routinely monitored using stringent quality control and quality assurance systems. Testing conducted on multiple batches confirms that the product complies with the established product specifications.

Tianrui Chemical has indicated that, because its *L-theanine* ($\geq 98\%$) ingredient is intended as an alternative to the Taiyo International and Blue California L-theanine ingredients already considered GRAS, and the conditions of use are identical, no major impact on the intake of L-theanine in the overall diet of the public would be expected. The present case simply constitutes

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an introduction into the market by another supplier who will have to compete in essentially the same markets and foods.

Based on the aforementioned considerations, it is my opinion that, by virtue of its substantial equivalence to two other L-theanine ingredients already considered GRAS for identical food uses, Tianrui Chemical's L-theanine ingredient [*L-theanine* ($\geq 98\%$)] would also be considered GRAS by qualified experts, making it exempt from definition as a *food additive* requiring premarket approval.

(b) (6)



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Zhejiang Tianrui Chemical Co., Ltd.
January 16, 2014

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SUBMISSION END

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